A STUDY ON SPECIES RELATIONSHIPS AND INHERITANCE OF CHARACTERS IN GENUS, SECTION, AND SUBSECTION LACTUCA L.

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWII IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN HORTICULTURE

DECEMBER 1992

BY

PATRICK J. O'MALLEY

DISSERTATION COMMITTEE:

RICHARD W. HARTMANN, CHAIRMAN
GERALD D. CARR
YONEO SAGAWA
JOSEPH DEFRANK
KENNETH Y. TAKEDA

We certify that we have read this dissertation and that, in our opinion, it is satisfactory in scope and quality as a dissertation for the degree of Doctor of Philosophy in Horticulture.

DISSERTATION COMMITTEE

Richard W. Hartmann
Chairman

Yoneo Sagawa

J. C. Brown

Kenneth A. Sorensen

ACKNOWLEDGMENTS

I would like to express my deep graditude to Dr. Richard W. Hartmann for his assistance throughout this research, and for his helpful advice in the preparation of this dissertation.

I would like to express my appreciation to the Horticulture Department for financial support as a research assistant.

I especially would like to thank my parents for the encouragement they have given me throughout my education.

Finally, I wish to give special thanks to my lovely wife Yunxia and my darling daughter Malinda who have inspired me to obtain this degree.

ABSTRACT

Interspecific crossability and F_1 hybrid vigor, chromosome pairing, pollen stainability, and achene fertility were used to assess relationships among *Lactuca aculeata* Boiss. & Kotschy, *L. altaica* Fisch. & Mey., *L. capensis* Thunb., *L. perennis* L., *L. saligna* L., *L. sativa* L., *L. serriola* L., and *L. virosa* L.

Lactuca sativa, *L. serriola*, *L. altaica*, and *L. aculeata* were fully intercompatible and belong in a species complex (*L. sativa-L. serriola*) which forms the core of *Lactuca* section *L.* subsection *L.* *Lactuca saligna* crossed with members of the *L. sativa-L. serriola* complex only when used as the female, some of the F_1's had abnormal growth, but all had meiotic irregularities, and lower pollen stainability and achene fertility. *Lactuca virosa* did not cross with *L. saligna*, but when used as the female did produce hybrids with the *L. sativa-L. serriola* complex. The F_1's had abnormal growth, many meiotic irregularities, and no pollen staining or achene fertility. Therefore, *L. virosa* is more distantly related to the *L. sativa-L. serriola* complex than is *L. saligna*. Neither *L. capensis* nor *L. perennis* crossed with any of the other species and are not in subsection *Lactuca*.

Previously unreported characters segregated within the *L. sativa-L. serriola* complex. Yellow pollen color was dominant to white giving 9:7 and 3:1 ratios caused by two complementary loci (*wp*-1 and *wp*-2). Basal branching was dominant to non-branching giving 3:1 and 13:3 ratios caused by a dominant allele for branching (*b*-1) at one locus

epistatic to a second locus with a dominant allele for non-branching (*b*-2). Extra lobe formation on leaf dorsal sides was caused by a new allele (U^a) at the leaf lobing locus which was dominant to both lobed (*U*) and unlobed (*u*). Bitterness was quantitative and segregated approximately 1/16 non-bitter suggesting at least two loci. Linkage was tested between the above loci and other loci for anthocyanin pigmentation, spines, achene color, leaf tip shape, and involucre position. The *b*-2 branching locus was linked with the leaf lobing locus and the locus for spines was linked with one anthocyanin locus.

Crosses between *L. saligna* and the *L. sativa-L. serriola* complex, also segregated for previously unreported characters. Branching segregated 13:3. Pappus bristle width segregated 3:1 two-cell width to one-cell width. Anthocyanic anther sheaths segregated three with anthocyanin to one without.

TABLE OF CONTENTS

Page

ACKNOWLEDGMENTS iii

ABSTRACT iv

LIST OF TABLES viii

LIST OF FIGURES x

LITERATURE REVIEW 1

Description of genus *Lactuca* 1
Origin and geographical distribution of genus *Lactuca* 2
Sections of genus *Lactuca* 2
Description of species in subsection Lactuca 3
Origin and importance of lettuce 4
Chromosome numbers in *Lactuca* 5
Interspecific crosses in subsection *Lactuca* 6
Genetic studies in lettuce 11

MATERIALS AND METHODS 16

Initial procedures 16
Plant material 16
Planting procedure 19
Crossing procedure 20
Procedure for analysis of species relationships of section *Lactuca* 21
Relationship study 21
Procedure for inheritance study in subsection *Lactuca* 28
Characters under investigation 29
Pollen color 29
Basal branching 29
Bitterness 30
Abnormal leaf growth 31
Pappus bristles 31
Anthocyanic anther sheaths 32
Achene beak length to body length ratio 32
Linkage detection 33

RESULTS AND DISCUSSION OF RELATIONSHIPS 34

Species identification 34
Status of crossing attempts 38
Crosses with *L. capensis* and *L. perennis* 38
Crosses between *L. sativa* and *L. serriola* 39
Crosses with originally mislabeled accessions with *L. sativa* and *L. serriola* 42

TABLE OF CONTENTS (Continued)

Crosses within *L. saligna* and between *L. sativa* and *L. saligna* and between *L. serriola* and *L. saligna* 46
Crosses between *L. sativa* and *L. virosa* and between *L. serriola* and *L. virosa* 51
Crosses between *L. saligna* and *L. virosa* 52
Crosses with *L. aculeata* 54
Crosses with *L. altaica* 58

SUMMARY AND CONCLUSIONS OF RELATIONSHIPS 59

RESULTS AND DISCUSSION OF INHERITANCE STUDY 63

Inheritance of new characters in *L. sativa*, *L. serriola*, and *L. aculeata* 63
Pollen color 63
Basal branching 66
Bitterness 70
Abnormal leaf growth 71
Inheritance of previously reported characters 73
Anthocyanin pigmentation 73
Spines 75
Leaf lobing 77
Reflexed involucre 79
Achene color 80
Leaf tip shape 81
Linkage 82
Inheritance of characters in crosses with *L. saligna* 89
Previously reported characters 89
Branching 90
Pappus bristles 92
Anthocyanic anther sheaths 93
Achene beak length to body length ratio 94

SUMMARY AND CONCLUSIONS OF INHERITANCE STUDY 97

LITERATURE CITED 100

LIST OF TABLES

Table		Page
1	Original species identifications of accessions of *Lactuca* species used	18
2	Attempted crosses	23
3	Correct species designation for *Lactuca* accessions	37
4	Crosses with *L. capensis* and *L. perennis* which did not produce hybrid achenes	38
5	Crosses with correctly labeled *L. sativa* and *L. serriola* accessions	40
6	Results of crosses between *L. sativa* and *L. saligna*, within *L. saligna*, and between *L. saligna* and *L. serriola*	48
7	Pollen fertility and achene fertility percentages of F_1 hybrids between *L. saligna* and *L. sativa*	48
8	Results of crosses between *L. sativa* and *L. virosa* and *L. serriola* and *L. virosa*	52
9	Results of crosses between *L. aculeata* and *L. sativa*, *L. serriola*, *L. saligna*, and *L. virosa*	55
10	Pollen color segregation in F_2 populations	64
11	F_3 segregation for pollen color	66
12	Branching habit of F_1's in greenhouse and field	67
13	Segregation for branching in F_2 populations	68
14	13:3 segregation for branching in F_2 populations	69
15	Bitterness segregation in F_2 populations	70
16	Segregation for abnormal leaf lobes in F_2 populations	72
17	Anthocyanin segregation in F_2 populations	74
18	Spine segregation in F_2 populations	76
19	Leaf lobing segregation in F_2 populations	78
20	Involucre segregation in F_2 populations	79

LIST OF TABLES (Continued)

Table		Page
21	Achene color segregation in F_2 populations	80
22	Leaf tip shape segregation in F_2 populations	81
23	Test for linkage between pollen color and other characters	83
24	Tests for linkage between branching and other characters	84
25	Linkage between leaf lobing and branching	85
26	Other character combinations tested for linkage	86
27	Linkage between anthocyanin pigmentation and spines	88
28	Segregation for branching in F_2 populations with *L. saligna*	91
29	Segregation for pappus bristle cell width in F_2 populations with *L. saligna*	92
30	Segregation for anthocyanic anther sheaths in F_2 populations with *L. saligna*	94
31	Average achene beak length, achene body length, and ratio between beak and body for parents and F_1's	95
32	Segregation for achene beak length to body length ratio in F_2 populations with *L. saligna*	96

LIST OF FIGURES

Figure		Page
1	Diakinesis in *L. sativa* (PI 491222)	41
2	Diakinesis in *L. serriola* (PI 491117)	41
3	Diakinesis in *L. serriola* x *L. sativa* hybrid (491117 x 'Valmaine')	42
4	Diakinesis in PI 281876 x *L sativa* (PI 491222)	44
5	Diakinesis in PI 273579 x *L sativa* (PI 183324)	44
6	Diakinesis in *L. saligna* (Ac 11-1)	47
7	Diakinesis in *L. saligna* x *L. sativa* (Ac 11-1 x 'Manoa')	47
8	Diakinesis in *L. saligna* x *L. sativa* (Ac 11-1 x 'Manoa')	50
9	Metaphase in *L. saligna* x *L. sativa* (PI 491208 x 'Manoa')	50
10	Diakinesis in *L. virosa* (PI 274375)	53
11	Diakinesis in *L. virosa* x *L. serriola* (PI 274375 x PI 491117)	53
12	Diakinesis in *L. aculeata* x *L. sativa* (Ac 3777 x PI 342517)	56
13	Metaphase in *L. serriola* x *L. aculeata* (PI 491117 x Ac 3777)	56
14	Diakinesis in *L. aculeata* (Ac 3777)	57
15	Diakinesis in *L. saligna* x *L. aculeata* (PI 491208 x Ac 3777)	57
16	Diakinesis in *L. altaica* (PI 289015) x PI 273574	58
17	Crossing diagram of *Lactuca* species used in this study	60

LITERATURE REVIEW

Description of genus Lactuca

Lactuca is a genus in the Compositae family originally described by Linnaeus (1752). The members of this genus are annual, biennial, or perennial herbs with abundant latex. They have leaves that are either glabrous, pubescent or prickly, are arranged spirally, and include two kinds, basal and stem. The basal leaves are petiolate, cauline, sessile, either undivided or pinnately lobed, and usually in a rosette. The stem leaves are usually bract-like, saggitate or hastate at the base, clasping, and often have auricles. The stem starts to lengthen after a variable period of rosette type growth and can be either glabrous or prickly, erect or ascending, simple or branched in the upper part, is 25-250 cm long, and develops into the inflorescence.

The inflorescence is a corymbose, pyramidal or spike-like panicle with numerous heads of 4-25 (exceptionally up to 50) ligulate florets. Each head has a cylindrical involucre 5-22 mm long with 3-4 imbricate rows of bracts. The green bracts are glabrous or hairy at the top and often violet tipped. The florets are longer than the involucre, and have a tube half as long as the ligule. The tube sometimes has a ring of long hairs at the top. The ligule has five teeth and may be yellow, yellow with a reddish tinge, blue, or rarely white. The anthers are fused into a tube which is yellow and has short appendages. The style is filiform and yellow, and forks outward at the tip.

The receptacle is flat and free of chaff. The achenes are compressed, generally fusiform, irregularly ribbed, beaked or unbeaked, 2.8-15 mm long by 1-2 mm wide, white, olive grey, or pale brown to black, and occasionally have winged margins. The beaks are short and stout, less than or equal to the body, and concolorous; or filiform, longer than the body, and paler. The achene is tipped with a white or yellowish uniseriate pappus 2.5-7 mm long. The individual setae are soft, not more than 4-celled at the base, and mostly deciduous. (Ferakova, 1977)

Origin and geographical distribution of genus Lactuca

Genus *Lactuca* originated in the northern hemisphere in warm temperate regions of the old world. The genus can now be found from sea level to 2500 m, but usually between 200-600 m in Europe (Ferakova, 1977; Hegi, 1929; Ross-Craig, 1963), North and South America (Britton, 1913; Gleason, 1952; Cronqvist, 1955; Abrams and Ferris, 1960; Radloff, 1961; Vuillemier, 1973), Africa (Stebbins, 1936,1937; Jeffrey, 1966; Tackholm, 1974), and Asia (Zoku, 1965; Jeffrey, 1975; Koster, 1976; Tuisl, 1977; and Shih, 1988). In Eurasia there is a general northern limit of 50-55^{o}N (Ferakova, 1977).

Sections of genus Lactuca

Genus *Lactuca* includes four sections: *Phaenixopus* (Cass.) Benth., *Mulgedium* (Cass.) C.B. Clarke, *Lactucopsis* (Sch.-Bip.) Rouy, and *Lactuca*; which are distinguished from each other by their achene characteristics (Babcock et al., 1937; Ferakova, 1977). Section

Lactuca differs from the other three *Lactuca* sections by having an achene with a distinct, usually filiform, beak at least as long as the body and of a different color (Ferakova, 1977).

Section *Lactuca* is further subdivided into 2 subsections: *Cyanicae* D.C. and *Lactuca* (Ferakova, 1977).

Subsection *Cyanicae* includes perennial species with capitula of 22 or less florets, blue ligules, and achenes with 1-3 ribs (Ferakova, 1977). This subsection contains the European species *L. perennis*, *L. intricata* Boiss., *L. tenerrima* Pourr., the African species *L. leptocephala* Stebbins (Ferakova, 1977) and probably the African species *L. capensis* and *L. kenyaensis* Stebbins (Babcock et al., 1937, Stebbins 1936).

Subsection *Lactuca* includes annual, winter annual, or biennial herbs with capitula of 10-30 (50) florets, yellow ligules, and achenes with many ribs. This subsection includes *L. sativa*, as well as *L. serriola*, *L. saligna*, *L. altaica*, *L. virosa*, *L. livida* (Ferakova, 1977) and possibly *L. aculeata* (Lindqvist, 1960a; Zohary, 1991) and *L. dregeana* (Lindqvist, 1960c).

Description of species in subsection Lactuca

The four main species in subsection *Lactuca* are *L. sativa*, *L. serriola*, *L. saligna*, and *L. virosa* (Lindqvist, 1960c). *Lactuca sativa*, an annual, has no prickles, erect involucre bracts, setose non-winged achenes, pappus bristle two cells wide, and an open panicle (Lindqvist, 1960c). *Lactuca serriola*, an annual or biennial, differs from *L. sativa* primarily by the presence of prickles on both the

midribs and stem and the reflexed involucre bracts (Lindqvist, 1960c; Ferakova, 1977). *Lactuca saligna*, an annual or perennial, differs from *L. serriola* by having fewer and softer prickles on the undersides of the midribs, few if any prickles on the stem, narrow leaves, spiculate achenes, pappus bristles one cell wide, and a spike-like panicle (Lindqvist, 1960c; Ferakova, 1977; Zohary, 1991). *Lactuca virosa*, a biennial, differs primarily from *L. serriola* by having much darker green leaves, and achenes with winged margins that are neither spiculate nor setose (Lindqvist, 1960c; Ryder, 1979; Zohary, 1991).

Several other species have been named in this subsection but Lindqvist (1960a, 1960c) and Ryder (1979) question their validity. *Lactuca aculeata* (Tuisl, 1968; Cohen and Liston, 1986; Zohary, 1991) is very similar to *L. serriola* except for having denser prickles on the midribs and stem, higher numbers of soft hairs on both sides of rigidly held leaves, and wider-angled panicle branches. *Lactuca altaica* (Lindqvist, 1960c; Ferakova, 1977; Zohary, 1991) is very similar to *L. sativa* except for having prickles on the underside of the midrib. *Lactuca dregeana* D.C. and *L. livida* Boiss. (Lindqvist, 1960c; Ferakova, 1977; Zohary, 1991) are very similar to *L. virosa*.

Origin and importance of lettuce

The only *Lactuca* species of commercial importance is *L. sativa* (Robinson et al., 1983). Ryder (1979) thinks lettuce originated along the Mediterranean sea coast, where a large diversity of lettuce types exists. He speculates that lettuce spread from the mild coastal regions to the harsher interior regions and evolved new ecotypes in

the process. In warmer areas slower bolting forms evolved which permitted maximum leaf development and better competitive ability, while in colder areas long-day flowering types evolved to insure reproduction before sub-viable temperatures occurred.

Lindqvist (1960c) lists three theories on the origination of cultivated lettuce; 1) it was derived from wild forms of *L. sativa*, 2) it originated directly from *L. serriola*, and 3) it originated from hybridization between different species in the subsection *Lactuca*.

The earliest recorded evidence of cultivated lettuce is from the tomb paintings of 4500 B.C. in Egypt. These depict narrow leaved plants which appear to be an early form of cos lettuce (Ryder, 1979). Lettuce was used by both the Greeks and Romans in the cos and leaf forms (Helm, 1954). Lettuce was introduced to China between 600-900 A.D. where the main part eaten is the stem (Helm, 1954). Head lettuce existed at least as far back as 1543 (Helm, 1954). Today lettuce is cultivated on all continents and in most countries of the world (Ryder, 1979) and is the most valuable fresh vegetable crop grown in the United States (Ryder, 1986) and fourth most valuable in Hawaii (Stat. of Hawaiian Ag., 1990).

Chromosome numbers in Lactuca

The somatic chromosome numbers that have been reported in *Lactuca* are 16, 18, 34 and 36 (Whitaker and Jagger, 1939; Thompson et al., 1941; Whitaker and Thompson, 1941; Thompson, 1943; Einset, 1944; Stebbins et al., 1953; Lindqvist, 1960A; Ferakova, 1977; Moore, 1965-1985; and Zohary, 1991).

Babcock et al. (1937) speculated that the original somatic number in *Lactuca* was either 16 or 18. However, Stebbins (1953) later concluded that the original number in the genus was 18, with early divergence towards 16.

Although section *Lactuca* has species with 16, 18, and 34 somatic chromosomes, the 16 and 34 chromosome species are all in subsection *Cyanicae*. All species classified as members of subsection *Lactuca* have 18 somatic chromosomes (Ferakova, 1977).

Interspecific crosses in subsection Lactuca

Lactuca has a high degree of autogamy that inhibits spontaneous cross-pollination either within species or between species (Stebbins, 1957). The only reported spontaneous interspecific cross in subsection *Lactuca* is *L. saligna* x *L. serriola*, listed under the name *Lactuca* x *dichotoma* Simk. (Ferakova, 1977).

The first report of an intentional cross in this subsection was between *L. serriola* and *L. sativa* by Durst (1930). He reported that the two species crossed easily in both directions and produced fertile hybrids. Since then several other researchers have also successfully crossed *L. serriola* and *L. sativa* without difficulty (Ernst-Schwarzenbach, 1936; Whitaker and Jagger, 1939; Whitaker and Thompson, 1941; Thompson et al., 1941; Lindqvist, 1960a; Vries, 1990). These two species are so closely related that Lindqvist (1960a) suggested that they belong to the same ecospecies.

The first attempts to cross *L. saligna* x *L. sativa* were unsuccessful in both directions (Thompson et al., 1941). However,

Thompson et al. (1941) and Brown and Michelmore (1988) were able to cross *L. saligna* with *L. serriola* and obtain partially fertile hybrids. They were unsuccessful with the reciprocal cross.

Lindqvist (1960a) studied many combinations of *L. saligna* with *L. serriola* and *L. sativa* and reported that these crosses were successful sometimes only when *L. saligna* was used as the female parent, and even then, only imperfectly developed hybrid seeds were obtained. Somatic chromosome doubling frequently occurred in the hybrids and some crosses produced dwarf hybrids.

Vries (1990) also was successful when he used *L. saligna* as a female parent in crosses with *L. serriola* and *L. sativa*. He obtained hybrids with very limited fertility in approximately one fourth of his parental combinations. When he used *L. saligna* to pollinate *L. serriola* he obtained only two hybrids from 42 combinations, and they had very low fertility. Likewise when he used *L. saligna* to pollinate *L. sativa* he obtained only one hybrid from 23 combinations, also with very low fertility.

Ernst-Schwarzenbach (1936) first reported an attempt to cross *L. virosa* with either *L. serriola* or *L. sativa*. The crosses were unsuccessful in both directions with no achenes produced. However, Thompson et al. (1941) did successfully cross *L. sativa* with *L. virosa*. They obtained vigorous hybrid plants, but all were completely sterile. The reciprocal cross failed completely. They were unsuccessful in crosses of *L. serriola* with *L. virosa* in either direction.

Thompson and Ryder (1961) were able to cross *L. virosa* as a female parent with an F_1 plant of *L. serriola* x *L. sativa*. They then applied colchicine to the infertile F_1 and obtained a partially fertile 4n. A *L. sativa* variety was then pollinated with pollen from the hybrid tetraploid. In the resulting F_1 there was a fertile 2n plant that had the leaf color and strong root system of *L. virosa*. After subsequent backcrosses to *L. sativa* and generation selection, a new variety named 'Vanguard' was created.

Lindqvist (1960a) conducted a comprehensive cytogenetic study with *L. virosa*, *L. serriola*, and *L. sativa*. He was able to make successful crosses between *L. virosa* and *L. serriola* and *L. sativa* in both directions, but the hybrid achenes were imperfectly developed. All crosses with cultivated *L. sativa* lines produced hybrids that died at an early stage. Crosses with *L. serriola* and other *L. sativa* lines gave viable hybrids, and in one case dwarfs, but no F_2 plants had the normal 18 chromosome number.

More recently, Eenink et al. (1982), crossed *L. sativa* x *L. virosa* and obtained apparently normal achenes, but the hybrids died as seedlings. They also reciprocally crossed *L. virosa* and *L. serriola* and obtained normal achenes. The F_1 plants showed hybrid vigor, but were male sterile. The hybrid plants did produce some viable seed when pollinated with pollen from *L. serriola*.

Vries (1990) crossed *L. sativa* x *L. virosa* and obtained achenes from 17 out of 25 combinations. Fourteen of the hybrids died at the rosette stage, while three produced vigorous sterile hybrids. In the reciprocal cross only one combination out of nine resulted in a hybrid

seed, but the plant died at the onset of flowering. He also crossed *L. virosa* x *L. serriola* and obtained hybrids in 15 combinations out of 43 attempts. All were sterile, except for one combination that had very limited fertility. The reciprocal cross yielded 21 hybrid combinations out of 34. Five of these had very limited fertility, including the one with the same parents that had very limited fertility in the *L. virosa* x *L. serriola* cross.

Matsumoto (1991) used somatic hybridization to cross *L. sativa* x *L. virosa*. About 20 plants that had more vigorous growth than either parent were confirmed as hybrids. However, the 2n chromosome number ranged from 28-53 (most were 2n=36) and all were sterile.

Lactuca saligna and *L. virosa* have never been successfully crossed in either direction (Thompson et al., 1941; Lindqvist, 1960a; Vries 1990)

Lindqvist (1960b) successfully crossed *L. altaica* and a line incorrectly labeled as *L. livida* with *L. sativa* and *L. serriola*. He considered *L. altaica* and the mislabeled line primitive forms of *L. sativa* because they are both intermediate in appearance between *L. serriola* and the more advanced *L. sativa*. In an earlier report, Thompson et al. (1941) said that *L. altaica* crossed easily in both directions to *L. sativa*. Lindqvist (1960c) suggested that the name *L. altaica* be used for all the species he considered to be primitive forms of *L. sativa*.

All attempts to cross species in subsection *Lactuca* with species outside the subsection have been unsuccessful (Thompson et al., 1941; Thompson, 1943) with the possible exception of the cross of *L.*

graminifolia, probably a species from subsection *Cyanicae* (Babcock et al., 1937; Lindqvist, 1960c), with *L. virosa* (Thompson et al., 1941). That cross produced one plant that was possibly a hybrid because even though it looked like the maternal parent, *L. graminifolia*, it had small patches of anthocyanin pigmentation on the upper surface of the leaf blade like the *L. virosa* paternal parent.

Thompson et al. (1941) attempted crosses with *L. perennis* from subsection *Cyanicae*. This species is regarded as having the closest relationship to subsection *Lactuca* (Kesseli and Michelmore, 1986). *Lactuca perennis* did not cross with *L. sativa*, *L. serriola*, or *L. virosa*. They did not attempt to cross *L. perennis* with *L. saligna*, which is the subsection *Lactuca* species that most resembles *L. perennis* morphologically.

In summary, *L. sativa* and *L. serriola* appear to be very closely related species. They cross easily in both directions and seem to have no barriers between them. They perform similarly in crosses with *L. saligna*, sometimes forming fertile hybrids with *L. saligna* as the female parent, but only very rarely when *L. saligna* is the male parent. However, *L. sativa* and *L. serriola* perform differently in crosses with *L. virosa*, forming only sterile hybrids with *L. sativa*, but occasionally forming partially fertile hybrids with *L. serriola*. Thus, there is some evidence to consider them separate species. *Lactuca virosa* and *L. saligna* are the most distantly related species in section *Lactuca*, since they have never been successfully crossed. *L. altaica* also crosses easily with *L. sativa* and *L. serriola* and may actually be a form of *L. sativa*.

Genetic studies in lettuce

In lettuce 65 morphological loci (Robinson et al., 1983; Ryder, 1983, 1988, 1989), 22 isoenzyme loci (Kesseli and Michelmore, 1986) and 143 restriction fragment length polymorphism loci (Kesseli et al., 1991) have been identified. The morphological loci include six that influence anthocyanin pigmentation, 13 for chlorophyll production, 12 for leaf morphology, six for heading and seedstalk formation, ten for flower and achene characteristics, seven for non-cytoplasmic male sterility, one for sensitivity to chemicals, and 13 for disease resistance.

Anthocyanin expression was originally reported by Durst (1930) to be caused by a single locus labeled *g* where the dominant allele *G* causes anthocyanin to be produced. Subsequently Ernst-Schwarzenbach (1936) determined anthocyanin was determined by two complementary genes, both of which must have a dominant allele for any anthocyanin to show in the leaves, stems, flower petals, and involucre bracts (Thompson, 1938). She retained the earlier named *g* for one gene and labeled the second gene *A*. Thompson (1938) also reported a dihybrid segregation caused by two complementary genes. He named the genes *T* and *C*, but his data suggest *T* and *g* are at the same locus. There are no reports of three gene segregations, so possibly *C* is also the same as the previously named *A* (Robinson et al., 1983).

There are three additonal loci whose effect can only be determined when anthocyanin is already present due to the forementioned genes. One of these determines the degree of anthocyanin pigmentation (Thompson, 1938; Lindqvist, 1960b). This

locus has four alleles. Listed in decreasing order of dominance they are *R*, red; R^{bs}, red-brown spotted; R^{s}, red-spotted; and R^{t}, red-tinged. Another locus causes an intensification of any existing anthocyanin pigmentation when the recessive allele *i* is present (Lindqvist, 1960b). The last locus has a recessive allele *v* which causes a plant that has genes for anthocyanin to fade as it gets older until it has lost all anthocyanin except on the spines and the dorsal side of the petals (Lindqvist, 1960b).

All 13 genes affecting chlorophyll production have an allele recessive to the normal dark green color and segregate 3:1 normal to mutant (Lindqvist, 1960b; Thompson, 1938; Ryder, 1965, 1971, 1975, 1983, 1989; Whitaker, 1944, 1968). It is likely that two of these genes are either tightly linked or are alleles at the same locus (Lindqvist, 1960b; Robinson et al., 1983).

The twelve leaf morphology genes can be subdivided into wax, hairs, venation, and shape. Glossy green leaves with thin wax covering (*gl*) are recessive to thick wax covering, which causes normal appearing dull grey-green leaves (Lindqvist, 1960b). Absence of prickles (*s*) is recessive to presence of prickles with an approximate 3:1 segregation (Durst, 1930). A locus for many abaxial leaf hairs (*lh*) was reported to be recessive or incompletely dominant to the no leaf hairs and to have a negative pleiotropic effect on sterility (Ryder, 1971). Striate parallel venation with tough leaves is caused by a single allele (*st*) recessive to normal netted venation (Whitaker and Bohn, 1953).

Leaf shape of the apex is controlled by a single gene with pointed leaves (*P*) dominant to rounded leaves (Lindqvist, 1960b). Six other leaf shape traits controlled by a single gene are: wavy, scalloped, leaf margins (*Sc*) dominant to highly serrated, frilly leaf margins; normal leaf type dominant to deeply indented, cut-leaf margins (*ct*); normal leaf type dominant to frilled, leathery, twisted leaves with protruding vascular bundles (*fr*); crinkled leaves with a blistered appearance (*Cr*) dominant to normal smooth leaves; normal leaf type dominant to angular dark green leaves on stunted sterile plants (*sn*); and normal leaf type dominant to strap-shaped leaves and highly frilled leaf margins (*en*) (Ryder, 1965, 1975). Leaf lobing was first reported as controlled by two complementary genes (Durst, 1930). A later report (Whitaker, 1950) asserts that segregation for leaf serration masked a single dominant gene for leaf lobing. A third report (Lindqvist, 1960b) postulates that one gene with three alleles (*U* lobed, U^{o} oak leaf, and *u* non-lobed) determines lobing, but that an undetermined linked gene affecting the gametophyte causes an excess of recessive non-lobed types.

There are three major recessive genes responsible for heading (*k, h, ca*) plus an undetermined number of modifying genes (Lindqvist, 1960b). Bolting under long days (*T*) is dominant to day neutral bolting response (Lindqvist, 1960b). There are two partially dominant genes that cause early flowering (Ef-1, Ef-2) (Ryder, 1983, 1988).

Lettuce flowers are normally yellow but there are three recessive genes causing salmon (*sg*), pale yellow (*pa*), and golden flowers (*go*) respectively (Ryder, 1971, 1989). The corolla normally has deep

clefts between the teeth but a recessive allele (*sh*) causes shallow clefts (Ryder, 1963a). Another recessive allele causes plump involucres (*pl*) instead of the normal tapered involucres (Ryder, 1971). An allele that causes the involucre bracts to bend backwards and expose the achenes to wind dispersal (*er*) is dominant to the normal nonreflexed (Whitaker and McCollum, 1954). Achene color is determined by two loci. At one locus an allele for yellow achene color (*y*) is recessive to dark brown. At the other locus an allele for white achene color (*w*) is recessive to dark brown and epistatic to yellow (Durst, 1930).

There are three complementary male sterility genes (*ms-1*, *ms-2*, *ms-3*). Pollen sterile plants result when all three of these loci are recessive (Lindqvist, 1960b). There are two other male sterility loci (*ms-4*, *Ms-5*) that show recessive-dominant epistasis which results in a 13:3 segregation in the F_2 for normal to male-sterile plants. These male-sterile plants can produce a few viable pollen grains (Ryder, 1963b). A sixth recessive male sterility gene (*ms-6*) causes nearly complete male sterility and partial female sterility (Ryder, 1967). A seventh male sterility gene (*Ms-7*) is dominant (Ryder, 1971).

Tolerance to the fungicide triforine (saprol) (*tr*) is recessive to susceptibility (Globerson and Eliasi, 1979; Smith, 1979).

There have been 13 genes identified for disease resistance, seven of which provide resistance to downy mildew (Johnson et al., 1977,1978; Zink and Duffus, 1970). There are three genes for resistance to lettuce mosaic virus (Ryder, 1970; Zink et al., 1973) and one gene each for resistance to bidens mosaic virus (Zitter and

Guzman, 1977), turnip mosaic virus (Zink and Duffus, 1970), and powdery mildew (Whitaker and Pryor, 1941).

MATERIALS AND METHODS

Initial procedures

Plant material

The plant materials used in this study were sampled from a collection of over 1000 *Lactuca* accessions that had been received from the Western Regional Plant Introduction Station, R. Provvidenti of Cornell University, E. J. Ryder of the U.S.D.A. in California, and various commercial seed companies. The primary focus was on species in section *Lactuca* subsection *Lactuca*. Representative accessions from all species of this subsection described by Ferakova (1977) (*L. altaica*, *L. livida*, *L. saligna*, *L. sativa*, *L. serriola*, and *L. virosa*) were included, even though Ferakova questioned the validity of *L. altaica* and *L. livida*. An accession labeled *L. dregeana*, which is a name introduced by DeCandolle (1838) for a South African species closely related to *L. virosa*, was also used. In addition, *L. aculeata*, described by Tuisl (1977) but unclassified for section and subsection, was included because Lindqvist (1960a) noted that it has many morphological features in common with *L. serriola*. Single accessions of *L. quercina* L. and of *L. squarrosa* (Thunb.) Miq., which both belong in a different section of *Lactuca*, were included because they had achenes very similar to *L. serriola*. *Lactuca perennis*, which belongs to the other subsection of *Lactuca*, *Cyanicae*, was included because it is the only member of *Cyanicae* known to have 18 somatic chromosomes and Kesseli and Michelmore (1986) stated that *L. perennis* is the closest species to the *Lactuca* subsection. *Lactuca*

capensis was included because Stebbins (1936) put this species in section *Lactuca* but did not indicate which subsection.

For species with less than three accessions available, all were planted. For others, a sample of up to nine representing the geographical diversity in the collection were planted. A special attempt to include accessions used by Kesseli and Michelmore (1986) was made because they questioned the validity of some of the identifications of their materials. Geographical diversity was used because accessions found at different locations would be more likely to differ in morphological characteristics. Table 1 lists the original species identification of the accessions used in this study, the source of achenes, and geographic origin.

Table 1. Original species identifications of accessions of *Lactuca* species used.

Species and Accession	Source	Origin
L. aculeata (ACU)		
Ac. #3777	R. Provvidenti	Turkey
L. altaica (ALT)		
PI 289015	W.R.P.I.S.[z]	Hungary
L. capensis (CAP)		
Ac. #3434	R. Provvidenti	Africa
L. dregeana (DRE)		
PI 273574[y]	W.R.P.I.S.	Italy
L. livida (LIV)		
Ac. #3980	R. Provvidenti	
PI 273585	W.R.P.I.S.	Denmark
L. perennis (PER)		
PI 271940	W.R.P.I.S.	Czechoslavakia
PI 273594	W.R.P.I.S.	Germany
PI 274378[y]	W.R.P.I.S.	France
L. quercina (QUE)		
Ac. #3006	R. Provvidenti	
L. saligna (SAL)		
Ac. #11-1	R. Provvidenti	Israel
Ac. #3789	R. Provvidenti	Turkey
PI 251798	W.R.P.I.S.	Italy
PI 253229[y]	W.R.P.I.S.	Turkey
PI 261653	W.R.P.I.S.	Portugal
PI 273582	W.R.P.I.S.	England
PI 281876	W.R.P.I.S.	Iraq
PI 491208	W.R.P.I.S.	Greece
L. sativa (SAT)		
Green Mignonette[x]	Locally increased seed	Hawaii
Mesa 659	Harris seed	California
Valmaine[y]	R.J. Ryder	California
Ac. #6002	R. Provvidenti	New York
PI 183324	W.R.P.I.S.	Egypt
PI 342517	W.R.P.I.S.	Netherlands
PI 491039	W.R.P.I.S.	Turkey
PI 491071	W.R.P.I.S.	Turkey
PI 491222	W.R.I.P.S.	Greece
L. serriola (SER)		
Ac. #3009	R. Provvidenti	New York
PI 190906[y]	W.R.I.P.S.	Czechoslavakia
PI 251245	W.R.P.I.S.	Egypt
PI 274372	W.R.P.I.S.	Russia
PI 274564	W.R.P.I.S.	Portugal
PI 491092	W.R.P.I.S.	Turkey
PI 491117	W.R.P.I.S.	
PI 491132	W.R.P.I.S.	

Table 1. (Continued) Original species identifications of accessions of *Lactuca* species used.

L. squarrosa (SQU)		
PI 236396	W.R.P.I.S.	Japan
L. virosa (VIR)		
Ac. #3350	R. Provvidenti	Romania
PI 271939[y]	W.R.P.I.S.	Portugal
PI 273579[y]	W.R.P.I.S.	Italy
PI 274375	W.R.P.I.S.	Poland

z Western Regional Plant Introduction Station
y Accessions used by Kesseli and Michelmore (1986)
x Grown in Hawaii under the name Manoa which it will be called in the remainder of this dissertation.

Planting procedure

Achenes were planted from June 1988 to October 1990. All achenes were germinated at 23 C in an air-conditioned laboratory [to prevent thermodormancy (Guedes and Cantliffe, 1980)] under 24-hours/day 40 watt cool flourescent tubes 15 cm from the surface in a mixture of one part peat moss and one part vermiculite. When the seedlings were about three cm in height (at approximately four weeks) the trays were placed in a greenhouse. When large enough (approximately seven weeks after planting) the plants were transplanted one per pot to pots 15 cm in diameter and 25 cm in depth containing the same medium used for germination. All plants were routinely fertilized every three weeks with a 10-30-10 liquid fertilizer.

Crossing procedure

Lettuce flowers are normally self-pollinated when the stigma picks up pollen as it grows through the anther sheath. To prevent self-pollination, this pollen must be removed before the stigma forks and bends outward. This was done by the washing method developed by Oliver (1910) and modified by Ryder (1974). Intermittent mist is applied during anther dehiscence to wash away the pollen grains so they cannot germinate on the receptive stigma. After drying, the stigma is then pollinated with pollen from another plant. This method of crossing produced 94% hybrid seed (Ryder, 1974).

Plants which were approaching flowering and were to be used as females were placed on a bench with intermittent mist. The mist nozzles were located above the lettuce flowers and were on for 15 seconds every five minutes. As the flowers opened (usually for only a couple of hours in the morning) the mist would wash the pollen off the stigmas. The lettuce plants were removed from the mist when the majority of the stigmas had emerged through the anther sheaths and had begun to fork outwards. Any flower heads whose stigmas had not emerged through the anther sheath, or were already closing, were removed. The flowers were dried with an electric fan prior to pollination with pollen from open flowers from the desired male line. An alternate emasculation method used was to gently wash the pollen off the emerging stigma with a fine stream of water from a water bottle every five minutes.

Pollination was made by rubbing the pollen-covered stigmas of an open flower head from the male parent over the stigmas of the

emasculated flower. After being pollinated, each flower head was tagged with the parental names and date of cross. From 1-16 flower heads on one female plant could be pollinated at one time. To detect if emasculation was effective 1-3 flower heads per plant were tagged but not pollinated. If a non-pollinated flower head on a plant produced achenes, selfing would have occurred and the parentage of the achenes produced by the other flowers on that plant on that date would be in doubt.

Procedure for analysis of species relationships of section Lactuca

Relationship study

Relationships of species were based on ability to produce hybrid achenes, the viability of the hybrid achenes, the ability of the hybrid plants to reach flowering stage, the nature of chromosome pairing in the hybrid pollen mother cells (PMCs), the pollen viability of the hybrids, and the frequency of viable achenes produced per flowerhead.

The accessions listed in Table 1 were planted from June 1988 to October 1990 and some grew well, while others grew poorly. Since there were many planting dates and various growth rates, crosses were made between whatever materials happened to be blooming on a particular day. Thus, some combinations were not obtained because the two parents did not ever flower on the same day. Crosses were attempted for all the combinations listed in Table 2.

For meiotic studies slides of PMCs were prepared by the procedure used by Carr (1976). Whenever possible chromosome behavior in at least 20 PMCs in diakinesis was examined for each hybrid combination.

An estimate of male fertility was conducted by pollen stains with cotton blue in lactophenol as outlined by Carr (1975). At least 100 pollen grains from each of five flower heads were counted for each plant examined.

Fertility estimates were made for all hybrid combinations that reached flowering stage. Achene fertility was estimated by the percentage of ovaries per mature head to form achenes, as used by Einset (1944). A minimum of ten heads per hybrid combination were scored.

Table 2. Attempted crosses.

Crosses with *L. aculeata*

ACU	3777	x	3350 VIR
ACU	3777	x	274375 VIR
ACU	3777	x	342517 SAT
ACU	3777	x	491208 SAL
VIR	3350	x	3777 ACU
SAT	Valmaine	x	3777 ACU
SAT	342517	x	3777 ACU
SER	491117	x	3777 ACU
SAL	491208	x	3777 ACU

Crosses with *L. altaica*

ALT	289015	x	491222 SAT
SAT	Manoa	x	289015 ALT
DRE	273574	x	289015 ALT

Crosses with *L. capensis*

CAP	3434	x	Manoa SAT
CAP	3434	x	274564 SER
CAP	3434	x	274372 SER
CAP	3434	x	491071 SAT
CAP	3434	x	491208 SAL
SAT	Manoa	x	3434 CAP
SER	274564	x	3434 CAP
SAT	491071	x	3434 CAP
SAL	491208	x	3434 CAP

Crosses with *L. dregeana*

DRE	273574	x	Manoa SAT
DRE	273574	x	190906 SER
DRE	273574	x	274378 PER
DRE	273574	x	289015 ALT
QUE	3006	x	273574 DRE
SAT	Manoa	x	273574 DRE
SAT	6002	x	273574 DRE

Crosses with *L. livida*

LIV	3980	x	183324 SAT
LIV	3980	x	274378 PER
LIV	3980	x	491092 SER
SAT	Manoa	x	3980 LIV
PER	274378	x	3980 LIV
SER	190906	x	3980 LIV

Table 2. (Continued) Attempted crosses.

Crosses with *L. perennis*

PER	274378	x	3980 LIV
PER	274378	x	253229 SAL
PER	274378	x	271939 VIR
PER	274378	x	274375 VIR
LIV	3980	x	274378 PER
SAL	251798	x	274378 VIR
SAL	253229	x	274378 PER
DRE	273574	x	274378 PER

Crosses with *L. quercina*

QUE	3006	x	273574 DRE
QUE	3006	x	273582 SAL
QUE	3006	x	281876 SAL
SAL	253229	x	3006 QUE
SAT	Manoa	x	3006 QUE
SAL	273582	x	3006 QUE
SAT	491222	x	3006 QUE

Crosses with *L. saligna*

SAL	11-1	x	Manoa SAT
SAT	Manoa	x	11-1 SAL
SER	274564	x	11-1 MAN
SAL	251798	x	274378 PER
SAL	253229	x	3006 QUE
SAL	253229	x	183324 SAT
SAL	253229	x	190906 SER
SAL	253229	x	273579 VIR
SAL	253229	x	274375 VIR
SAL	253229	x	274378 PER
SAL	253229	x	491208 SAL
SAL	253229	x	491222 SAT
SAT	Manoa	x	253229 SAL
SER	190906	x	253229 SAL
VIR	274375	x	253229 SAL
PER	274378	x	253229 SAL
SAT	491071	x	253229 SAL
SAL	261653	x	491208 SAL
SAL	273582	x	3006 QUE
SAL	273582	x	281876 SAL
QUE	3006	x	273582 SAL

Table 2. (Continued) Attempted crosses.

Crosses with *L. saligna* (Continued)

SAL 281876	x	273579 VIR
SAL 281876	x	274378 PER
SAL 281876	x	491092 SER
SAL 281876	x	491208 SAL
SAL 281876	x	491222 SAT
SAT Manoa	x	281876 SAL
QUE 3006	x	281876 SAL
SAL 273582	x	281876 SAL
SAT 491071	x	281876 SAL
SER 491117	x	281876 SAL
SAL 491208	x	Manoa SAT
SAL 491208	x	3350 VIR
SAL 491208	x	3434 CAP
SAL 491208	x	3777 ACU
SAL 491208	x	236396 SQU
SAL 491208	x	274372 SER
SAL 491208	x	274375 VIR
SAT Manoa	x	491208 SAL
QUE 3006	x	491208 SAL
CAP 3434	x	491208 SAL
ACU 3777	x	491208 SAL
SAL 253229	x	491208 SAL
SER 274372	x	491208 SAL
VIR 274375	x	491208 SAL
SER 274564	x	491208 SAL
SAL 281876	x	491208 SAL
SAT 491071	x	491208 SAL

Crosses with *L. sativa*

SAT Manoa	x	Valmaine SAT
SAT Manoa	x	3006 QUE
SAT Manoa	x	3434 CAP
SAT Manoa	x	3980 LIV
SAT Manoa	x	11-1 SAL
SAT Manoa	x	190906 SER
SAT Manoa	x	253229 SAL
SAT Manoa	x	273574 DRE
SAT Manoa	x	281876 SAL
SAT Manoa	x	289015 ALT
SAT Manoa	x	342517 SAT
SAT Manoa	x	491092 SER
SAT Manoa	x	491208 SAL
CAP 3434	x	Manoa SAT
SAL 11-1	x	Manoa SAT

Table 2. (Continued) Attempted crosses

Crosses with *L. sativa* (Continued)

SQU 236396 x Manoa SAT
DRE 273574 x Manoa SAT
SAL 491208 x Manoa SAT

SAT Manoa x Valmaine SAT
SAT Valmaine x 3777 ACU
SER 491117 x Valmaine SAT

SAT 6002 x 273574 DRE

SAT 183324 x 273579 VIR
LIV 3980 x 183324 SAT
SAL 253229 x 183324 SAT

SAT Manoa x 342517 SAT
SAT 342517 x 3777 ACU
SAT 342517 x 274375 VIR
ACU 3777 x 342517 SAT
VIR 274375 x 342517 SAT

SAL 281876 X 491039 SAT

SAT 491071 x 281876 SAL
SAT 491071 x 236396 SQU
SAT 491071 x 253229 SAL
SAT 491071 x 491208 SAL
CAP 3434 x 491071 SAT

SAT 491222 x 3006 QUE
SAT 491222 x 3777 ACU
SAL 253229 x 491222 SAT
ALT 289015 x 491222 SAT

Crosses with *L. serriola*

SER 3009 x 274375 VIR
VIR 274375 x 3009 SER

SER 190906 x 3980 LIV
SER 190906 x 253229 SAL
SER 190906 x 281876 SAL
SER 190906 x 491092 SER
SAT Manoa x 190906 SER
SAL 253229 x 190906 SER
DRE 273574 x 190906 SER

Table 2. (Continued) Attempted crosses.

Crosses with *L. serriola* (Continued)

SER 251245 x 236396 SQU
SQU 236396 x 251245 SER
VIR 273579 x 251245 SER

SER 274564 x 3434 CAP
SER 274564 x 11-1 SAL
SER 274564 x 491208 SAL
SER 274564 x 274372 SER

SER 274372 x 236396 SQU
VIR 271939 x 274372 SER
SER 274564 x 274372 SER
SAL 491208 x 274372 SER

SER 491092 x 183324 SAT
SAT Manoa x 491092 SER
SER 190906 x 491092 SER
SAL 281876 x 491092 SER

SER 491117 x Valmaine SAT
SER 491117 x 3777 ACU
SER 491117 x 281876 SAL

Crosses with *L. squarrosa*

SQU 236396 x Manoa SAT
SQU 236396 x 251245 SER
SER 251245 x 236396 SQU
VIR 273579 x 236396 SQU
SER 274372 X 236396 SQU
SAT 491071 x 236396 SQU
SAL 491208 x 236396 SQU

Crosses with *L. virosa*

VIR 3350 x 3777 ACU
ACU 3777 x 3350 VIR
SAL 491208 x 3350 VIR

VIR 271939 x 274372 SER
VIR 271939 x 491071 SAT
PER 274378 x 271939 VIR

VIR 273579 x 236396 SQU
VIR 273579 x 251245 SER
SAT 183324 x 273579 VIR

Table 2. (Continued) Attempted crosses.

Crosses with *L. virosa* (Continued)

SAL 253229 x 273579 VIR
SAL 281876 x 273579 VIR

VIR 274375 x 3009 SER
VIR 274375 x 253229 SAL
VIR 274375 x 342517 SAT
VIR 274375 x 491117 SER
VIR 274375 x 491208 SAL
SER 3009 x 274375 VIR
ACU 3777 x 274375 VIR
SAL 253229 x 274375 VIR
PER 274378 x 274375 VIR
SAL 281876 x 273579 VIR
SAT 342517 x 274375 VIR
SAL 491208 x 274375 VIR

Procedure for inheritance study in subsection Lactuca

Among the materials used for investigating species relationships a number of characters not previously reported in the literature were noticed. The inheritance of these characters was studied by growing F_2 segregating populations of crosses differing in expression of these characters.

Crosses were made as previously described. Putative hybrid achenes were grown in the greenhouse along with the parents to confirm their hybrid nature. Achenes from confirmed F_1 plants were saved and grown at the Poamoho research farm on Oahu to examine the F_2 character segregation. Attempts were made to grow at least 200 individual plants for each segregating population. When necessary, F_3 populations were also grown. Chi square tests were used to determine significance of genetic ratios and for the detection of linkage.

Characters under investigation

Pollen color

There are no previous reports describing differences of pollen color in section *Lactuca*. All the accessions examined had yellow pollen grains (Y) with the exception of one accession (PI 281876, labeled *L. saligna*) which had white pollen grains (W). The following crosses were made to study the inheritance of this character:

PI 281876 W x PI 273579 Y

PI 281876 W x PI 274378 Y

PI 281876 W x PI 491092 Y

PI 281876 W x PI 491222 Y

Ac 3006 Y x PI 281876 W

Manoa Y x PI 281876 W

PI 273582 Y x PI 281876 W

PI 491071 Y x PI 281876 W

Basal branching

Inheritance of basal branching has not been previously reported. Basal branching is a weedy characteristic found in all the *L. saligna* and *L. serriola* accessions, but rarely in *L. sativa*. Accessions were classified by whether they had single stems (S) or branched stems (B) near the soil line. The following crosses were made to study the inheritance of this character:

Manoa S x PI 253229 B

Manoa S x PI 281876 B

Manoa S x PI 491092 B

Valmaine S x Ac 3777 B

PI 273582 S x PI 281876 B

PI 491071 S x PI 281876 B

Ac 11-1 B x Manoa S

Ac 3777 B x PI 342517 S

PI 236396 B x Manoa S

PI 253229 B x PI 183324 S

PI 253229 B x PI 273579 S

PI 281876 B x PI 273579 S

PI 491092 B x PI 183324 S

PI 491117 B x Valmaine S

PI 491208 B x Manoa S

Bitterness

Commercially grown lettuce (*L. sativa*) such as Manoa has no acrid or bitter taste. Most PI accessions, especially those not from *L. sativa*, have an extremely bitter or acrid taste. The following crosses between bitter (B) and non-bitter (N) accessions were evaluated for this character.

Manoa N x PI 190906 B

Manoa N x PI 281876 B

Abnormal leaf growth

One accession (Ac. #3006, labeled as *L. quercina*) has an abnormal leaf lobe character in which extra lobes originate on both sides of the dorsal midrib where it branches into the first lobe. Inheritance of this characteristic has not been previously reported. The following crosses between Ac 3006 with abnormal leaf (A) and accessions with normal leaves (N) were made to study the inheritance of this character:

PI 491222 N x Ac 3006 A

Manoa N x Ac 3006 A

PI 253229 N x Ac 3006 A

PI 273582 N x Ac 3006 A

Ac 3006 A x PI 273574 N

Ac 3006 A x PI 273582 N

Ac 3006 A x PI 281876 N

Pappus bristles

The pappus bristles of all accessions examined, except those from *L. saligna*, include both two-cell and one-cell width bristles in approximately equal frequency. All the accessions of *L. saligna* had bristles only one-cell wide (Both *L. perennis* and *L. capensis* have bristles three cells wide). Ferakova (1977) used this characteristic to separate *L. saligna* from other members of the subsection. The following crosses between *L. saligna* accessions with one-cell width pappus bristles (S) and the other species with two-cell width pappus bristles (D) were made to study the inheritance of this character:

Ac 11-1	S x Manoa	D
PI 491208	S x Manoa	D
PI 491208	S x Ac 3777	D
PI 491208	S x PI 236396	D

Anthocyanic anther sheaths

Besides anthocyanin expression in the leaves and petals, all the *L. saligna* lines also had anthocyanic anther sheaths while all other accessions had no anthocyanin in the anther sheaths. The following crosses between accessions with anthocyanic anther sheaths (A) and with normal yellow sheaths (Y) were made to study the inheritance of this character:

Ac 11-1	A x Manoa	Y
PI 491208	A x Manoa	Y
PI 491208	A x Ac 3777	Y
PI 491208	A x PI 236396	Y

Achene beak length to body length ratio

The actual characteristic under investigation is the ratio of the achene beak length to the achene body length. Inheritance of this character has not been previously reported. This characteristic was used by Lindqvist (1960c) to help differentiate *L. saligna* from *L. sativa* and *L. serriola*. In the accessions used as parents, ratios ranged from 2:1 for one line of *L. saligna* (PI 491208) to

approximately 1:1 for the one accession of *L. aculeata* (Ac. #3777) and all of the *L. serriola* and *L. sativa* lines (except for PI 190906 and Ac. #6002). Sometimes the ratios varied within lines. The following crosses were made between high beak ratios (H) and low beak ratios (L) lines:

Ac 11-1 H x Manoa L

PI 491208 H x Manoa L

PI 491208 H x Ac 3777 L

PI 491208 H x PI 236396 L

Linkage detection

All the crosses listed above were tested for linkage to other characters that were segregating, and to each other. Other characters that were segregating include anthocyanin pigmentation (presence or absence), involucre position (reflexed or nonreflexed), spination (presence or absence), leaf lobing (lobed or entire), and leaf shape (pointed or round). Linkage in the F_2 populations was detected by X^2 for linkage. Contingency tables were used for disturbed segregations (Mather, 1951) and linkage intensities were estimated by the product method (Immer, 1930).

RESULTS AND DISCUSSION OF RELATIONSHIPS

Species identification

Several of the *Lactuca* accessions used in this study were received with incorrect species identifications. The species identifications of all accessions were determined by comparison to taxonomic keys (Lindqvist, 1960c; Ferakova, 1977) for achenes and for plant characteristics from the seedling through the achene ripening stages.

The accession labeled *L. dregeana*, PI 273574, did not have black achenes with wing margins on each side as expected for this species (Ferakova, 1977; Lindqvist, 1960c). Each achene of PI 273574 had five to ten ribs on each side, a white filiform beak, and a white body characteristic of *L. sativa* in subsection *Lactuca* (Ferakova, 1977). Plants grown from achenes of this accession had no spines on leaf midribs or stems confirming that it is *L. sativa*.

The one accession labeled *L. livida* did not have black achenes with wing margins on each side as has been reported for *L. livida* (Ferakova, 1977). The accession, Ac 3980, had each achene with five to ten ribs on each side, a white filiform beak, and a dark brown body characteristic of subsection *Lactuca* (Ferakova, 1977). Plants grown from achenes of this accession had no spines on leaf midribs or stems confirming that it also is *L. sativa*.

Lactuca perennis in section *Lactuca* subsection *Cyanicae* is perennial and has achenes with one to three ribs on each side (Ferakova, 1977). PI 273594 fit this description, but PI 274378 had

each achene with five to ten ribs on each side as well as other achene characters of subsection *Lactuca*, and was annual. Whether this accession should be placed in *L. sativa* or *L. serriola* is uncertain because it has spines on the leaf midribs and stems as in *L. serriola*, but has the non-reflexed involucre of *L. sativa*.

Lactuca quercina in section *Lactucopsis* should have achenes with five to eight ribs on each side and a black body which narrows into a black beak (Ferakova, 1977). Ac 3006, labeled as *L. quercina*, however, had each achene with five to ten ribs on each side, a white filiform beak, and a brown body characteristic of species in subsection *Lactuca* (Ferakova, 1977). Whether this accession should be placed in *L. sativa* or *L. serriola* is uncertain because it has spines on the leaf midribs and stems as in *L. serriola*, but has the non-reflexed involucre of *L. sativa*. This accession, however, does have oak leaf type leaves as reported for *L. quercina*.

Three accessions of *L. saligna* had achenes that were a little larger with a beak to body ratio lower than normally found in *L. saligna*. When they were grown out, all three accessions had unlobed leaves and a panicle type inflorescence in contrast to *L. saligna*, which has lobed leaves and a spike type inflorescence. For one of these accessions, PI 273582, it is uncertain whether this accession should be placed in *L. sativa* or *L. serriola* because it has spines on the leaf midribs and stems as in *L. serriola*, but has the non-reflexed involucre of *L. sativa*. The other two accessions, PI 253229 and PI 251798, had no spines and were classified as *L. sativa*. A fourth accession of *L. saligna*, PI 281876, had achenes similar to those

normally found in *L. saligna*, but when this accession was grown out it had spines on both the leaf midribs and stems and a panicle type inflorescence and was classified as *L. serriola*. Ac 11-1, PI 261653, and PI 491208 were correctly labeled as *L. saligna*.

Lactuca squarrosa, possibly of section *Lactuca* (Babcock et al., 1937), should have achenes which are black with winged margins, one to three ribs on each side, and a thick short beak (Shih, 1988). Each achene of the accession labeled *L. squarrosa*, PI 236396, had five to ten ribs on each side, a white filiform beak, and a dark brown body with no winged margins characteristic of subsection *Lactuca* (Ferakova, 1977). Plants grown from achenes of this accession had no spines on the leaf midribs or stems which puts them in *L. sativa*.

Two accessions of *L. virosa* did not have black achenes with winged margins on each side as has been reported for *L. virosa* (Ferakova, 1977). Both these accessions had achenes characteristic of other species of subsection *Lactuca*. Plants grown from achenes of PI 273579 were uncertain for placement beacuse they had spines on the leaf midribs and stems as in *L. serriola*, but nonreflexed involucres like *L. sativa*, while plants grown from achenes of PI 271939 had no spines on the leaf midribs or stems which places them in *L. sativa*. Ac 3350 and PI 274375 were correctly labeled *L. virosa*.

The correct species classification for each accession is listed in Table 3. The originally mislabeled accessions were kept in this study to confirm their species identification, to determine their relationships to the other species in subsection *Lactuca*, and to contribute characters for the morphological diversity study.

Table 3. Correct species designation for *Lactuca* accessions.

Original label	Accession	Correct species name
aculeata	Ac. #3777	aculeata
altaica	PI 289015	altaica
capensis	Ac. #3434	capensis
dregeana	PI 273574	sativa
livida	Ac. #3980	sativa
perennis	PI 273594	perennis
	PI 274378	serriola or sativa
quercina	Ac. #3006	serriola or sativa
saligna	Ac. #11-1	saligna
	PI 251798	sativa
	PI 253229	sativa
	PI 261653	saligna
	PI 273582	serriola or sativa
	PI 281876	serriola
	PI 491208	saligna
sativa	Manoa	sativa
	Valmaine	sativa
	Ac. #6002	sativa
	PI 183324	sativa
	PI 342517	sativa
	PI 491039	sativa
	PI 491071	sativa
	PI 491222	sativa
serriola	Ac. #3009	serriola
	PI 190906	serriola
	PI 251245	serriola or sativa
	PI 274372	serriola
	PI 274564	serriola
	PI 491092	serriola
	PI 491117	serriola
squarrosa	PI 236396	sativa
virosa	Ac. #3350	virosa
	PI 271939	sativa
	PI 273579	serriola or sativa
	PI 274375	virosa

Status of crossing attempts

Crosses with L. capensis and L. perennis

None of the eleven crosses involving *L. capensis*, or the eight crosses involving *L. perennis* produced achenes (Table 4). This confirms a previous report (Ferakova, 1977) that *L. perennis* is in a different subsection (*Cyanicae*) and is not compatible with species of subsection *Lactuca*. Since *L. capensis* did not cross with any other species, and had a chromosome number of n=8 (subsection *Lactuca* has n=9), it too does not belong in subsection *Lactuca*.

Table 4. Crosses with *L. capensis* and *L. perennis* which did not produce hybrid achenes.

Crosses with *L. capensis*

CAP	3434	x	Manoa SAT
CAP	3434	x	274372 SER
CAP	3434	x	274375 VIR
CAP	3434	x	274564 SER
CAP	3434	x	491071 SAT
CAP	3434	x	491208 SAL
SAT	Manoa	x	3434 CAP
VIR	274375	x	3434 CAP
SER	274564	x	3434 CAP
SAT	491071	x	3434 CAP
SAL	491208	x	3434 CAP

Crosses with *L. perennis*

PER	273594	x	11-1 SAL
PER	273594	x	Manoa SAT
PER	273594	x	274375 VIR
PER	273594	x	491117 SER
SAL	11-1	x	273594 PER
SAT	Manoa	x	273594 PER
VIR	274375	x	273594 PER
SER	491117	x	273594 PER

Crosses between L. sativa and L. serriola

Crosses between accessions originally correctly labeled as *L. sativa* or *L. serriola* (Table 5) all produced F_1 plants. *Lactuca sativa* (Figure 1) and *L. serriola* (Figure 2) parents had normal growth and normal meiosis with nine bivalents, two of them associated with the nucleolus. *Lactuca sativa* x *L. serriola* hybrids also had normal meiosis (Figure 3). F_1's between *L. sativa* x *L. serriola* all had 95% or greater pollen staining and 88% or greater achene fertility, even higher than some of the *L. sativa* x *L. sativa* and *L. serriola* x *L. serriola* hybrids. Thus, there were no compatibility differences between these two species. Surprisingly SAT Manoa had the lowest achene fertility (62%) in this group. This may be because the heat of the greenhouse (somtimes in excess of 40 C) may have affected the highly selected SAT Manoa more adversely than the 'weedy' accessions. SAT 342517 is a butterhead type of lettuce similar to SAT Manoa and it too had a lower achene fertility (81%).

Table 5. Crosses with *L. sativa* and *L. serriola* accessions.

Cross	Pollen staining %	Achene fertility %
Crosses within *L. sativa*		
SAT Manoa x Valmaine SAT	93	88
SAT Manoa x 342517 SAT	90	83
Crosses between *L. sativa* and *L. serriola*		
SAT Manoa x 190906 SER	98	88
SAT Manoa x 491092 SER	96	98
SER 491117 x Valmaine SAT	95	100
Crosses within *L. serriola*		
SER 190906 x 491092 SER	93	95
SER 274564 x 274372 SER	94	91

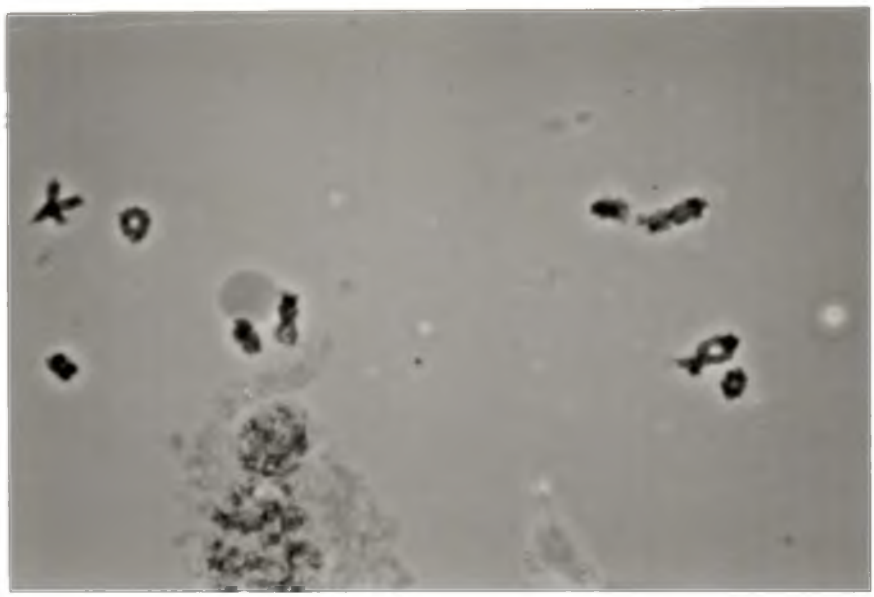

Figure 1. Diakinesis in *L. sativa* (PI 491222). Nine bivalents, X 1000.

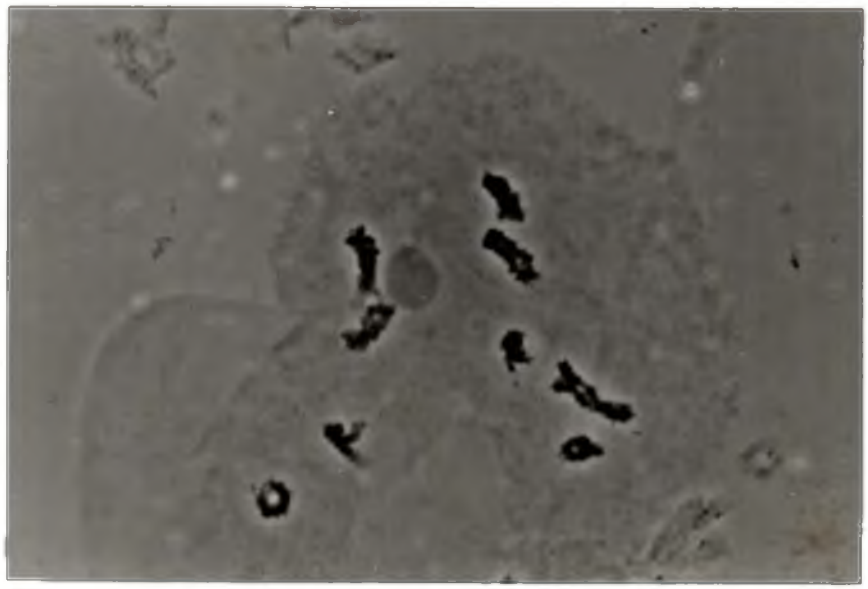

Figure 2. Diakinesis in *L. serriola* (PI 491117). Nine bivalents, X 1200.

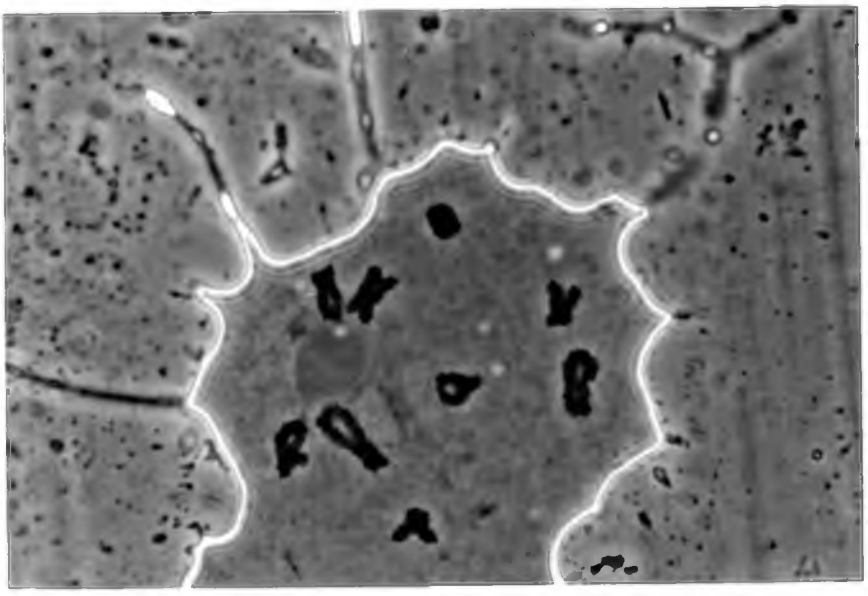

Figure 3. Diakinesis in *L. serriola* x *L. sativa* hybrid (491117 x 'Valmaine'). Nine bivalents, X 1200.

Crosses with originally mislabeled accessions with L. sativa and L. serriola

273574, originally received as *L. dregeana*, in crosses with SAT 6002, SAT Manoa, and SER 190906 had 97, 94, and 95% pollen staining, and 91, 95, and 79% achene fertility respectively, and meiosis was normal (see Figures 4,5 for meiosis in a similar type of cross). Thus, 273574 not only looks like L. sativa, but behaves in crosses like it, also.

3980 was originally received as *L. livida*. In crosses with SAT 183324, and SER 190906, the F_1's had 93 and 96% pollen staining, 98 and 96% achene fertility and meiosis was normal (see Figures 4,5). Thus, 3980 also looks like and behaves like *L. sativa*.

274378, originally received as *L. perennis*, was crossed with the now confirmed SAT 3980. The F_1 had 97% pollen staining, 98% achene fertility and normal meiosis (see Figures 4,5). Thus, 274378 is either *L. sativa* or *L. serriola* and not *L. perennis*.

3006, originally received as *L. quercina*, was crossed with SAT Manoa and SAT 491222. The F_1's had 92 and 96% pollen staining, and 94 and 94% achene fertility and normal meiosis (see Figures 4,5). Thus, 3006 also is either *L. sativa* or *L. serriola*.

273582, originally received as L. saligna, when crossed with the above SAT-SER 3006, had 92% pollen staining, 97% achene fertility and normal meiosis (see Figures 4,5). Thus, 273582 also appears to be *L. sativa* or *L. serriola*.

253229, also originally received as *L. saligna*, was crossed with SAT Manoa, SAT 491222, and SER 190906. The F_1's had 94, 93, and 98% pollen staining, 84, 95, and 99% achene fertility, and normal meiosis (see Figures 4,5). Thus, 253229 looks and behaves like another *L. sativa* accession.

251798, another accession originally received as *L. saligna*, was crossed with 274378 which is either *L. sativa* or *L. serriola*. The F_1 had only 68% pollen staining, but 96% achene fertility and normal meiosis (see Figures 4,5). Thus, 251798 behaves as well as looks like *L. sativa*.

281876 was also originally received as *L. saligna*. In crosses with SAT Manoa, SAT 491222, SAT 491071, SER 491092, and SER 491117 the F_1's had 96, 93, 91, 91, and 96% pollen staining, and 94, 96, 99, 99, and 98% achene fertility and normal meiosis (Figure 4). Thus, 281876

Figure 4. Diakinesis in PI 281876 x *L sativa* (PI 491222). Nine bivalents, X 1200.

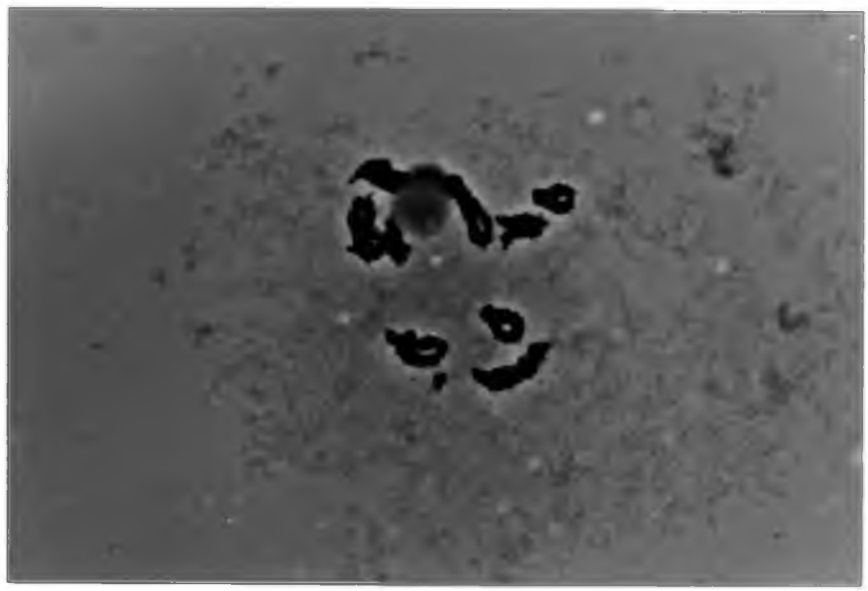

Figure 5. Diakinesis in PI 273579 x *L sativa* (PI 183324). Nine bivalents, X 1200.

also behaves like *L. serriola*.

236396, originally received as *L. squarrosa*, was crossed with SAT Manoa, and SAT 491071. The F_1's had 96 and 94% pollen staining, 98 and 97% achene fertility, and normal meiosis (see Figures 4,5). Thus, 236396 both looks like and behaves like *L. sativa*.

273579, originally received as *L. virosa*, was crossed with SAT 183324. The F_1 had 94% pollen staining, 96% total fertility, and normal meiosis (Figure 5). Thus, 273579 is either *L. sativa* or *L. serriola*.

271939, also originally received as *L. virosa*, was crossed with 274378 which behaves like *L. sativa* or *L. serriola*. The F_1 had 91% pollen staining, 95% achene fertility, and normal meiosis (see Figures 4,5). Thus, 271939 looks and behaves like *L. sativa*.

Thus, all 11 of the originally mislabeled accessions performed in crosses as if they are either *L. sativa* or *L. serriola*. Of the 11 originally mislabeled accessions, six fit the characteristics of *L. sativa* (236396, 273574, 3980, 251798, 253229, and 271939), one fits all the characteristics of *L. serriola* (281876), and four were classified as *L. serriola-L. sativa* (3006, 273579, 273582, and 274378) because they had spines like *L. serriola*, but non-reflexed involucres like *L. sativa*.

Besides the four accessions relabeled *L. serriola-L. sativa* that had characters of both species, an additional accession labeled *L. serriola* (251245) was very heterogeneous and had plants with spines with both reflexed and non-reflexed involucres, as well as plants with

no spines and reflexed and non-reflexed involucres. This accession was also relabeled *L. serriola-L. sativa*.

Both the spines and reflexed involucre bracts (allows wind dispersal of the achenes) as found in *L. serriola* are undesirable characters for a cultivated species. Distinguishing between the wild *L. serriola* and the cultivated *L. sativa* based solely on genetically inherited morphological characters (see section on old characters) that can easily be transferred between the two species seems somewhat artificial. This suggests that *L. sativa* is a cultivated form of *L. serriola*.

Crosses within L. saligna and between L. sativa and L. saligna and between L. serriola and L. saligna

The *L. saligna* parents and the two intraspecific *L. saligna* crosses had normal growth and normal meiosis (Figure 6). Like *L. sativa* and *L. serriola* they had nine bivalents with two associated with the nucleolus. Pollen staining and achene fertility were both above 90% (Table 7).

In crosses between *L. sativa* and *L. saligna* only the three crosses involving *L. saligna* as the female were successful (Table 6). All the hybrid plants were at least as large as the smaller parent with the exception of SAL 491208 x SAT 236396 which was a semi-dwarf (50 cm) one-half the size of the smaller (100 cm) SAT parent. All the hybrid plants reached flowering stage.

In the F_1 hybrid SAL 11-1 x SAT Manoa 76% of the cells had nine bivalents (Figure 7). Often, one or two of the bivalents would appear

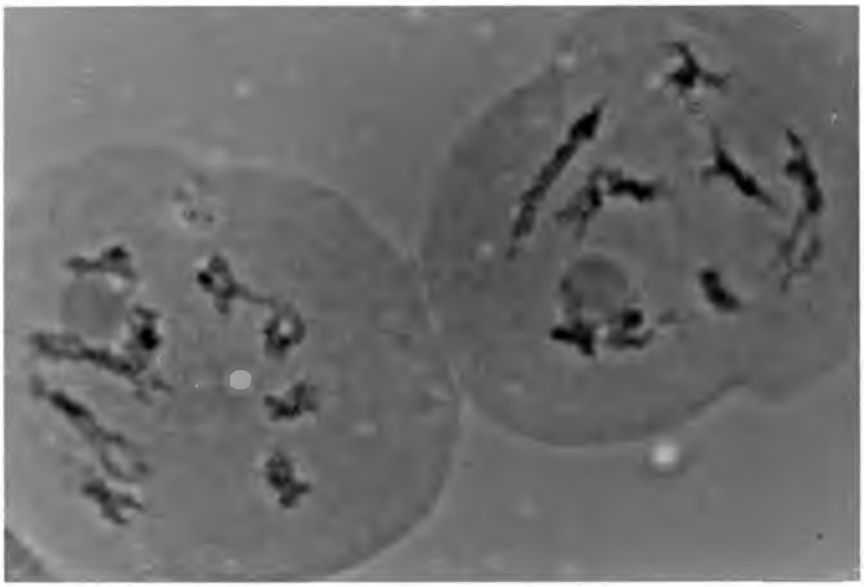

Figure 6. Diakinesis in *L. saligna* (Ac 11-1). Nine bivalents, X 1200.

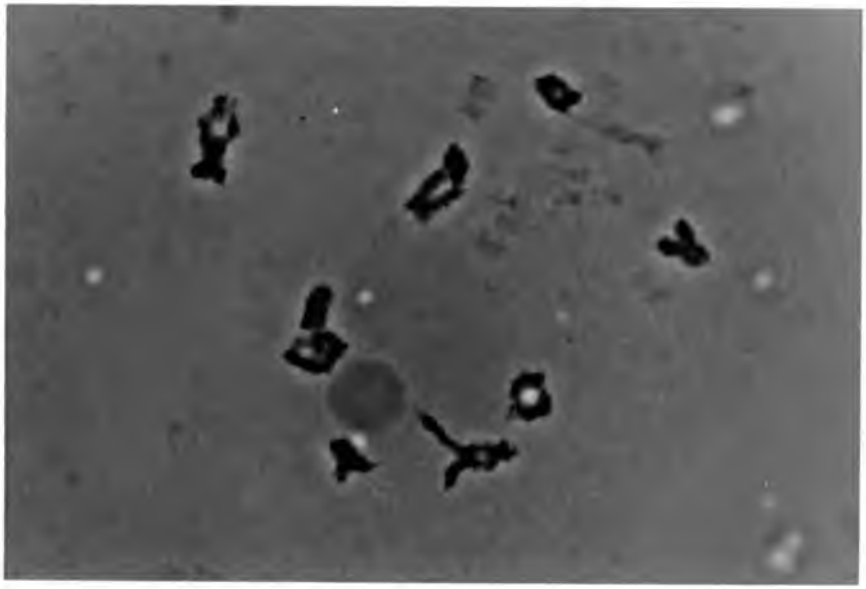

Figure 7. Diakinesis in *L. saligna* x *L. sativa* (Ac 11-1 x 'Manoa'). Nine bivalents, X 1200.

Table 6. Results of crosses between *L. sativa* and *L. saligna*, within *L. saligna*, and between *L. saligna* and *L. serriola*.

Crosses between *L. sativa* and *L. saligna*

SAL	11-1	x	Manoa SAT	Y[z]
SAT	Manoa	x	11-1 SAL	N
SAL	491208	x	Manoa SAT	Y
SAL	491208	x	236396 SAT	Y
SAT	Manoa	x	491208 SAL	N
SAT	491071	x	491208 SAL	N

Crosses within *L. saligna*

SAL	11-1	x	261653 SAL	Y
SAL	261653	x	491208 SAL	Y

Crosses between *L. saligna* and *L. serriola*

SER	274564	x	11-1 SAL	N
SAL	491208	x	274372 SER	N
SER	3006	x	491208 SAL	N
SER	274372	x	491208 SAL	N
SER	274564	x	491208 SAL	N

z Listed as hybrid plants produced Yes (Y) or hybrid plants produced No (N)

Table 7. Pollen fertility and achene fertility percentages of F_1 hybrids between *L. saligna* and *L. sativa*.

Cross	Female parent		Male parent		F_1 hybrid	
	Pollen	Achene	Pollen	Achene	Pollen	Achene
SAL 11-1 x Manoa SAT	96	94	94	62	59	1.5
SAL 491208 x Manoa SAT	91	96	94	62	34	0.1
SAL 491208 x 236396 SAT	91	96	97	74	28	0.1

only loosely connected, forming rod bivalents (Figure 8), suggesting segmental rather than complete homology. The other 24% of the cells had univalents. The F_1 hybrid of SAL 491208 x SAT Manoa had 35% of the cells with complete pairing. Loosely paired bivalents occurred, as did univalents (Figure 9).

In all hybrids the pollen staining and achene fertility were lower than in the parents (Table 7). However, the hybrid SAL 11-1 x SAT Manoa had nearly twice as many pollen grains stained and 15 times as many achenes produced as SAL 491208 x SAT Manoa indicating that SAL 11-1 might have a closer relationship to *L. sativa* than SAL 491208.

There were no successful crosses between *L. serriola* and *L. saligna* (Table 6). However, only one cross used *L. saligna* as the female parent, the direction that resulted in all three crosses between *L. sativa* x *L. saligna*. This cross, SAL 491208 x SER 274372, was only tried two times, as compared to the cross of SAL 491208 x SAT Manoa which was attempted at least 15 times, but only produced hybrid achenes in three. Perhaps more attempts with *L. saligna* as the female parent, especially SAL 11-1, might have given some successful crosses. This suggests that female *L. serriola* crossed to *L. saligna* is either a very difficult or an incompatible cross, just like female *L. sativa* crossed to *L. saligna*.

Figure 8. Diakinesis in *L. saligna* x *L. sativa* (Ac 11-1 x 'Manoa'). Nine bivalents, note reduced chiasma frequency and increased number of rod bivalents, X 1200.

Figure 9. Metaphase in *L. saligna* x *L. sativa* (PI 491208 x 'Manoa'). Eight bivalents with one univalent in the upper left, and another in the lower right, X 1200.

Crosses between L. sativa and L. virosa and between L. serriola and L. virosa

There were no successful crosses between *L. sativa* and *L. virosa* out of four attempts (Table 8). However, two crosses between *L. serriola* and *L. virosa* that used *L. virosa* as the female were successful. The two crosses that used *L. virosa* as the male were not. One plant was obtained from the cross of VIR 274375 x SER 3009. It was about two-thirds the height (60 cm) of the smaller (80 cm) VIR parent, and it did flower. Four plants were obtained from the cross of VIR 274375 x SER 491117. One died in the seedling stage, one was a dwarf (20 cm), and the two others equaled the samaller VIR parent in size. However, only one of the latter produced flowers.

VIR 274375 had normal meiosis, also with nine bivalents, two associated with the nucleolus (Figure 10). However, a few cells had some late separating chromosomes at the first division not seen in the other species. The hybrids of VIR 274375 x SER 3009 and VIR 274375 x SER 491117 both had multiple univalents in all cells examined (Figure 11). It was difficult to determine configurations in these cells due to the large number of univalents and the overlapping of chromosomes, but it appears three loosely associated rod pairs and 12 univalents were fairly common. Both F_1's showed only 2-3 lightly stained pollen grains (the parents had > 90% darkly stained grains) indicating almost complete male sterility. No F_2 achenes were produced, but shriveled achenes were produced when the hybrids were pollinated with a different *L. serriola* accession. The results of these few crosses

Table 8. Results of crosses between *L. sativa* and *L. virosa* and *L. serriola* and *L. virosa*.

Crosses between *L. sativa* and *L. virosa*

VIR	274375	x	253229 SAT	N[z]
VIR	274375	x	342517 SAT	N
SAT	253229	x	274375 VIR	N
SAT	342517	x	274375 VIR	N

Crosses between *L. serriola* and *L. virosa*

VIR	274375	x	3009 SER	Y
VIR	274375	x	491117 SER	Y
SER	3009	x	274375 VIR	N
SER	274378	x	274375 VIR	N

z Listed as hybrid plants produced Yes (Y) or hybrid plants produced No (N).

seem to indicate that *L. serriola* and *L. virosa* are more closely related than *L. sativa* and *L. virosa*.

The two *L. virosa* accessions were not crossed because they flowered at different times. *Lactuca virosa* did not grow well under the warm temperatures of Hawaii.

Crosses between L. saligna and L. virosa

All three crosses between *L. saligna* and *L. virosa* (SAL 491208 x VIR 3350, VIR 274375 x SAL 491208, and SAL 491208 x VIR 274375) were unsuccessful. *L. saligna* and *L. virosa* are clearly different from *L. serriola* and *L. sativa* and each other.

Figure 10. Diakinesis in *L. virosa* (PI 274375). Nine bivalents, X 1200.

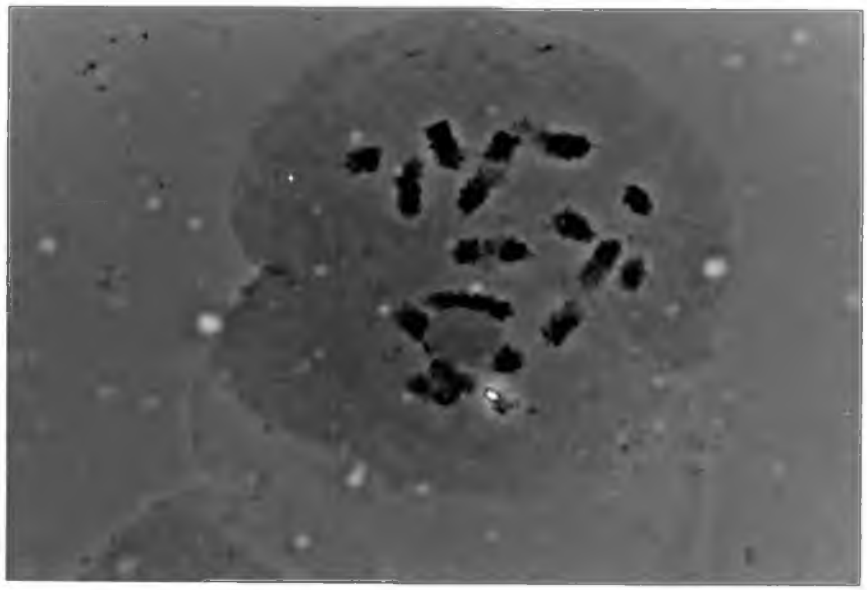

Figure 11. Diakinesis in *L. virosa* x *L. serriola* (PI 274375 x PI 491117). Eighteen univalents (possibly some weakly associated chains of two), X 1200.

Crosses with L. aculeata

Eight crosses were attempted between *L. aculeata* and other species (Table 9). Two crosses with *L. sativa* in both directions were successful, as was the one cross with *L. serriola*. A cross with *L. saligna* was successful when the *L. saligna* accession was the female parent, but not when it was the male parent. Three crosses using *L. virosa* were not successful in either direction. All hybrids with *L. sativa* and *L. serriola* had normal growth and normal meiosis (Figures 12,13) like in *L. aculeata* itself (Figure 14) or in *L. sativa*, *L. serriola*, and their hybrids (Figures 1-5). The F_1's all flowered and had high pollen staining percentages (94-98%) and high achene fertility (82-92%)(Table 9).

The F_1 between *L. saligna* and *L. aculeata* had normal growth. In diakinesis, 32% of the cells had complete pairing, but often with loosely associated chromosomes (Figure 15) as seen in *L. saligna* x *L. sativa* crosses. Twenty-two% of the cells had univalents, and there were a few cells that were possibly tetraploid. Lindqvist (1960a) observed some tetraploid cells in crosses between *L. saligna* and *L. sativa*, so the presence of tetraploid cells in this hybrid might also be posssible.

These results show that *L. aculeata* acted the same in all crosses with *L. sativa*, *L. serriola*, *L. virosa*, and *L. saligna* as *L. sativa*. Morphologically *L. aculeata* is more similar to *L. serriola* because it shares the characters of spines on both the midribs and stem, and reflexed involucres. When one of the crosses between *L. virosa* and *L. serriola* (VIR 274375 x SER 491117) was pollinated with pollen from

Table 9. Results of crosses between *L. aculeata* and *L. sativa*, *L. serriola*, *L. saligna*, and *L. virosa*.

Crosses with *L. aculeata*			Pollen fertility %	Achene fertility %
ACU 3777	x 3350 VIR	N[z]	-	-
ACU 3777	x 274375 VIR	N	-	-
ACU 3777	x 342517 SAT	Y	98	82
ACU 3777	x 491208 SAL	N	-	-
VIR 3350	x 3777 ACU	N	-	-
SAT Valmaine	x 3777 ACU	Y	94	92
SER 491117	x 3777 ACU	Y	96	90
SAL 491208	x 3777 ACU	Y	25	0.1

z Listed as hybrid plants produced Yes (Y) or hybrid plants produced No (N).

another *L. serriola* (pollen was not avalable from SER 491117), *L. sativa*, and *L. aculeata*, only shrivelled achenes were produced except with the pollen from *L. aculeata*. This suggests that *L. aculeata* may be closely related to *L. serriola* and particularly SER 491117. However, it differs from *L. serriola* by having unlobed rounded leaves (most *L. serriola* have lobed leaves), denser prickles on midribs and stem, higher numbers of soft hairs on both sides of the leaves, a longer period in the rosette stage, and wide angled panicle branches. Thus it is clearly a distinct entity, but since there are no incompatibility barriers between it and *L. sativa* and *L. serriola*, perhaps it should be a subspecies of one or the other of them (probably *L. serriola*), or a subspecies of a complex of *L. sativa* and *L. serriola*.

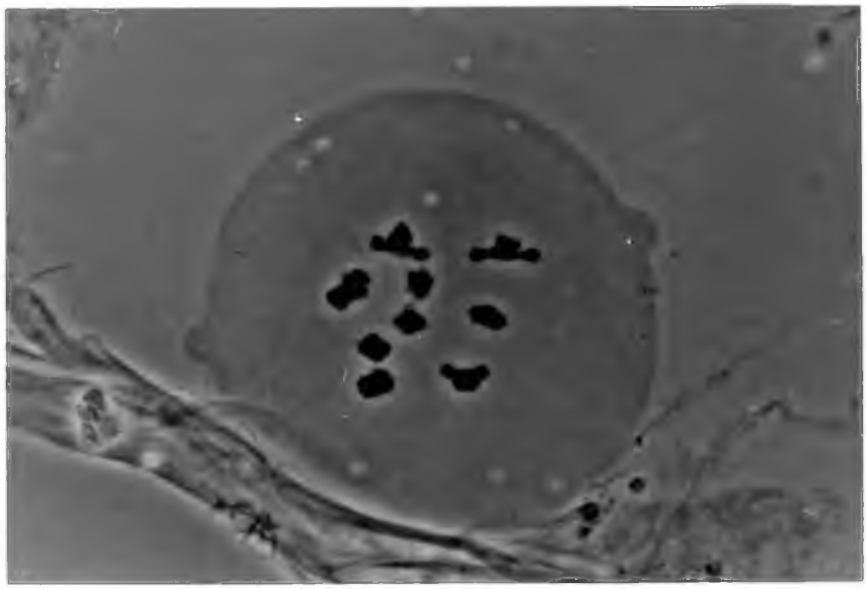

Figure 12. Diakinesis in *L. aculeata* x *L. sativa* (Ac 3777 x PI 342517). Nine bivalents, X 1200.

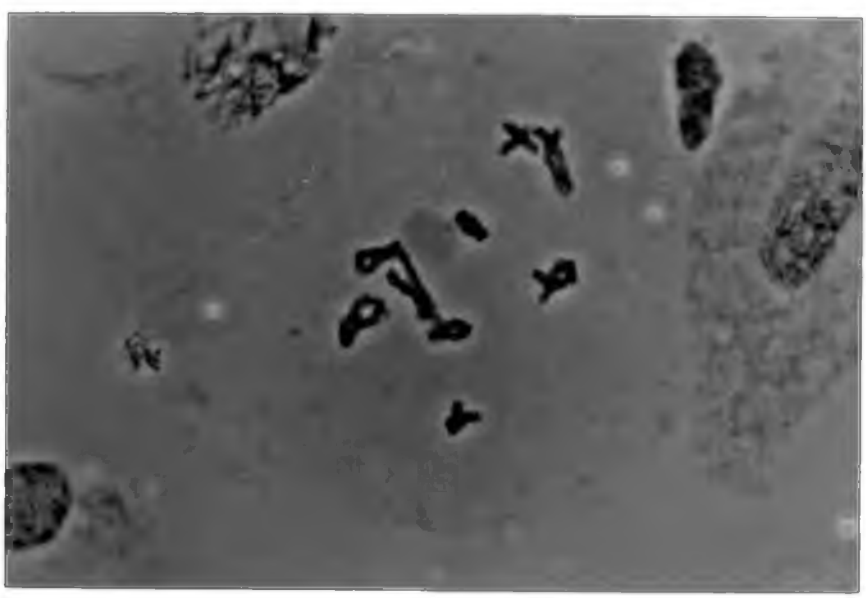

Figure 13. Metaphase in *L. serriola* x *L. aculeata* (PI 491117 x Ac 3777). Nine bivalents, X 1200.

Figure 14. Diakinesis in *L. aculeata* (Ac. 3777). Nine bivalents, X 1200.

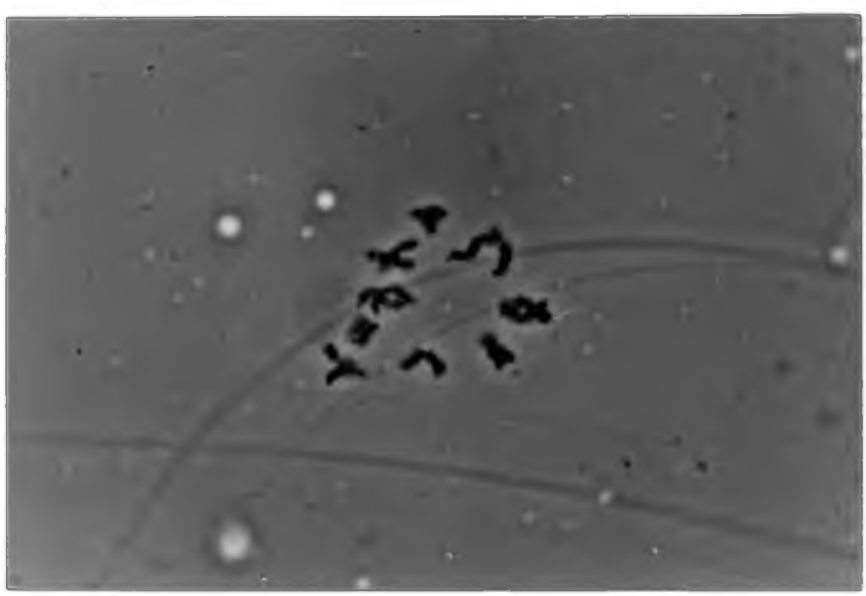

Figure 15. Diakinesis in *L. saligna* x *L. aculeata* (PI 491208 x Ac 3777). Note reduced chiasma frequency and increase in rod bivalents, X 1200.

Crosses with L. altaica

Only two crosses were attempted with *L. altaica* (ALT 289015 x SAT 491222 and SAT 273574 x ALT 289015). Both were successful and the F_1's had normal growth and normal meiosis as in *L. sativa*, *L. serriola*, *L. aculeata*, and their hybrids (Figure 16). The F_1's flowered and had 96 and 93% pollen staining, and 98 and 96% achene fertility. Thus, *L. altaica* seems closely related to *L. sativa*, and by extrapolation also to *L. serriola* and *L. aculeata*. The accession of *L. altaica* used in this study (ALT 289015, only one in the PI collection) is intermediate morphologically between *L. sativa* and *L. serriola* and does not have any distinct characters that are not found in either species. Therefore, I do not think it should be considered a valid species.

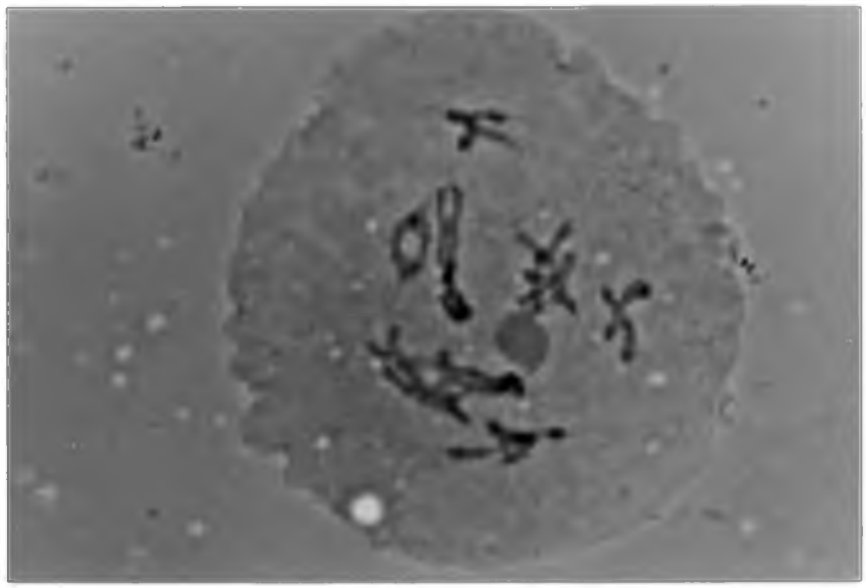

Figure 16. Diakinesis in *L. altaica* (PI 289015) x PI 273574. Nine bivalent, X 1200.

SUMMARY AND CONCLUSIONS OF RELATIONSHIPS

Based on these crossing relationships, *L. sativa, L. serriola, L. altaica,* and *L. aculeata* are a very closely related group with normal bivalent pairing, and should probably be considered one species. This is exemplified by the one *L. altaica* accession (289015), the four accessions classified as *L. sativa* or *L. serriola* (3006, 273579, 273582, and 274378) and the one *L. serriola* accession (251245) that share traits from *L. sativa*, *L. serriola*, and *L. aculeata*.

Lactuca saligna is more distantly related, but can still be crossed with members of this group when used as the female parent to give partially fertile hybrids. However, in diakinesis there is reduced chiasma frequency and sometimes presence of univalents. *Lactuca virosa* is more distantly related, crossed only with *L. serriola*, only when used as a female, had multiple univalents in diakinesis, and gave no fertile hybrids. *Lactuca saligna* and *L. virosa* are most distantly related and did not cross with each other, but genes could probably be transfered between these two species by using members of the first group as bridge species. *Lactuca perennis* and *L. capensis* did not cross with any of the other species and therefore should not be included in subsection *Lactuca*. Relationships are summarized in Figure 17.

The two most distinct members of the group of four closely related species are *L. aculeata*, a long day plant with dense prickles on the midribs and stem, high numbers of soft hairs on both sides of the leaves, wide angled panicle branches, and reflexed involucres, and

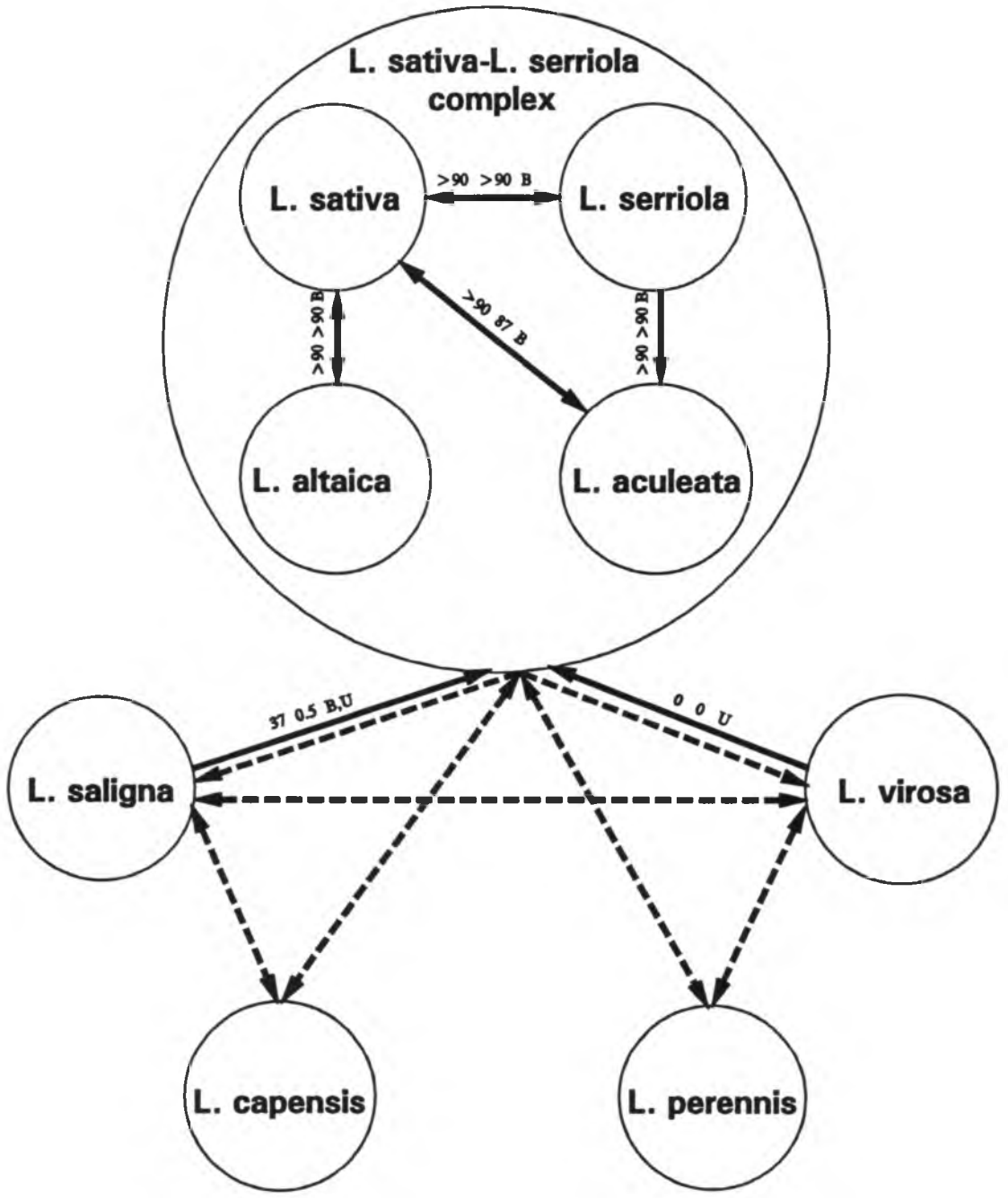

Figure 17. Crossing diagram of *Lactuca* species used in this study. Solid lines indicate crosses that produced hybrids, dashed lines indicate unsuccessful crosses, arrows point toward male parent. Numbers on lines are pollen staining and achene fertility percentages respectively. B is for only bivalents formed and U is for at least some univalents formed.

L. sativa, a day neutral plant with no spines or hairs, narrow angled panicle branches, and non-reflexed involucre bracts. *Lactuca serriola* and *L. altaica* are intermediate between these two species. *Lactuca serriola* is a long day plant with prickles on the midrib and stem, some soft hairs on both sides of the leaves, narrow angled panicle branches, and reflexed involucre bracts. *Lactuca altaica* is a day neutral plant with spines on the midribs, no soft hairs on either side of the leaves, narrow angled panicle branches, and non-reflexed involucre bracts. *Lactuca aculeata* and *L. sativa*, although distinct for most characters, do share two characters not usually found in *L. serriola* or *L. altaica*; prolonged rosette type growth and entire rounded leaves.

It is interesting that in the F_2 population of the cross between SAL 491208 (narrow leaf) x ACU 3777, plants resembling all species of subsection *Lactuca* except *L. virosa* were seen, including several plants that looked very similar to SAL 11-1 which is a wider leaf form of *L. saligna*, and is the accession that had higher fertility levels when crossed with *L. sativa*. This further suggests that the characters used to separate *L. sativa, L. serriola, L. aculeata,* and *L. altaica* may be simple genic differences and not sufficient to separate them as different species. It also leads to speculation that there could have been an earlier cross between *L. saligna* (narrow leaf) and *L. aculeata* that could have been the origination of *L. serriola, L. altaica, L. sativa*, and the wide leaf form of *L. saligna*. *Lactuca saligna* would have contributed lobed leaves, reduced spines, absence of hairs, and less angled panicles as found in *L. serriola, L.*

altaica, and *L. sativa*, while *L. aculeata* would have contributed wider leaves, more spines, and thicker stems with less branching to the wider leaf form of *L. saligna* as seen in SAL 11-1. It is also possible that *L. virosa* may have played some part in the make-up of these species, but its possible role cannot be determined at this time.

RESULTS AND DISCUSSION OF INHERITANCE STUDY

Inheritance of new characters in L. sativa, L. serriola, and L. aculeata

In the previous section it was shown that *L. sativa, L. serriola*, and *L. aculeata* are fully interfertile with no compatibility barriers between them. Therefore, in this section on inheritance of characters within this group, all references to species will be omitted as irrelevant.

Pollen color

Ten crosses were made with two plants of 281876, the only accession with white pollen. All F_1 plants had yellow pollen indicating yellow pollen is dominant. In the F_2 populations, two kinds of ratios were obtained, depending on which 281876 plant was used (Table 10) The two plants were labeled "a" and "b". Whether 281876 was used as the male or female seemed to have no effect.

Of the three populations from crosses with 281876a, two fit a 3:1 ratio very well, while the third (281876a x 274378) did not. Pooled, however, the fit to a 3:1 ratio was very good. F_3 populations of 281876a x 274378 were grown from 12 F_2 plants with yellow pollen and two with white pollen. All progeny of the white pollen F_2's were white. Of the yellow parent F_2's, ten populations segregated and two did not. When the 12 progeny of each F_2 parent were pooled and tested for a fit to the 5:1 ratio expected, the fit was very good (Table 11).

Table 10. Pollen color segregation in F_2 populations.

Yellow pollen parent	White pollen parent	Number of plants Observed (Expected) Yellow		White		Ratio	X^2	*P*
3006	281876a	90	(94.5)	36	(31.5)	3:1	0.85	.30-.50
273579[z]	281876a	90	(93.8)	35	(31.3)	3:1	0.60	.30-.50
274378[z]	281876a	44	(36.8)	5	(12.3)	3:1	5.72	.01-.02
Manoa	281876b	89	(79.9)	53	(62.1)	9:7	2.38	.10-.20
273582	281876b	91	(83.8)	58	(65.2)	9:7	1.41	.20-.30
274378[z]	281876b	41	(39.4)	29	(30.6)	9:7	0.15	.50-.70
491071	281876b	47	(42.2)	28	(32.8)	9:7	1.25	.20-.30
491092[z]	281876b	75	(74.8)	58	(58.2)	9:7	0.001	.95-.99
491117	281876b	43	(45.0)	37	(35.0)	9:7	0.21	.50-.70
491222[z]	281876b	48	(46.1)	34	(35.9)	9:7	0.18	.50-.70
Pooled crosses with 281876a		224	(225.0)	76	(75.0)	3:1	0.02	.80-.90
Pooled crosses with 281876b		434	(411.2)	297	(319.8)	9:7	2.89	.05-.10

Test for heterogeneity crosses with 281876a — *P*

- Total X^2 (3 df) = 7.17
- Pooled X^2 (1 df) = 0.02
- Heter. X^2 (2 df) = 7.15 — .02-.05

Test for heterogeneity crosses with 281876b — *P*

- Total X^2 (7 df) = 5.58
- Pooled X^2 (1 df) = 2.89
- Heter. X^2 (6 df) = 2.69 — .80-.90

z Line used as male parent.

The seven crosses with 281876b all gave a good fit to a 9:7 ratio (Table 10). F_3 populations were again grown from 12 F_2 plants with yellow pollen and two with white pollen, this time from the cross of 281876b x 491222. Both white populations did not segregate. Two yellow populations did not segregate, three seemed to segregate 3:1, and seven seemed to segregate 9:7. The 12 plants per F_3 population were too few to clearly separate these types, but when combined, they fit the expected 25:11 ratio (Table 11).

Thus, in 281876, the only accession with white pollen, a character not previously reported in *Lactuca*, there were plants with a recessive gene for this character at one locus as well as plants with recessive genes at two loci. The proposed names and symbols for these genes following gene nomenclature rules in lettuce (Robinson et al., 1983) are white pollen-1 *wp*-1, and white pollen-2 *wp*-2. 281876a has *wp*-1, and 281876b has *wp*-1 and *wp*-2.

Table 11. F_3 segregation for pollen color.

Cross	Number of plants Observed (Expected) Yellow	White	X^2 5:1[z]	P
281876b x 274378	114 (115.8)	25 (23.2)	0.17	.50-.70
281876a x 491222	110 (119.2)	33 (23.8)	4.27	.02-.05*
Cross			X^2 25:11[y]	
281876b x 274378	114 (96.5)	25 (42.5)	10.38	<.01**
281876a x 491222	110 (99.3)	33 (43.7)	3.77	.05-.10

z When F_2 segregates 3:1, 5:1 is the expected ratio for total F_3 population grown only from dominant phenotype F_2 plants.
y When F_2 segregates 9:7, 25:11 is the expected ratio for total F_3 population grown only from dominant phenotype F_2 plants.

Basal branching

Basal branching is a character that may not be fully expressed if conditions are not suitable for vigorous, unimpeded growth. Lines that would normally show branching in the field, often would not show branching in a pot in the greenhouse. Six of fourteen F_1 plants grown in pots did not show any branching, although in the field the F_2 segregations clearly showed that branching was dominant. Likewise, when one of the F_2 populations was planted two weeks later than the others in an end row that had untilled soil, unlike the other rows with well tilled soil, it was the only F_2 population with more unbranched than branched individuals. It was not included in the determination of segregation. Despite the variability in pot grown F_1's, 281876 showed branching in all four F_1 populations (Table 12).

Table 12. Branching habit of F_1's in greenhouse and field.

Cross	F_1 in greenhouse	F_1 in field
Manoa S[z] x 253229 B[y]	unbranched	branched
Manoa S x 281876 B	branched	branched
Manoa S x 491092 B	unbranched	-
Valma. S x 3777 B	unbranched	-
273582 S x 281876 B	branched	-
236396 B x Manoa S	unbranched	-
281876 B x 273579 S	branched	-
281876 B x 491222 S	branched	-
491117 B x Valma. S	branched	-

z Unbranched parent.
y Branched parent.

In the F_2 all populations had more branched than unbranched plants (Table 13). All but three crosses fit a 3:1 ratio. All three had 281876 as one of the parents and more branched plants than expected. A fourth cross with 281876 fit a 3:1 ratio, but also had a slight deficiency for unbranched plants.

The pooled X^2 for all crosses did not fit a 3:1 ratio. However, if all four crosses with 281876 were removed, the remaining five populations gave a very good fit for a 3:1 ratio and low heterogeneity indicating they came from one population which has a dominant gene that causes branching.

The four populations excluded from the 3:1 segregation were tested for a 13:3 ratio (Table 14). All populations fit a 13:3 ratio. The cross with 281876 that fit the 3:1 ratio also fit the 13:3 ratio at the same probability level, but had a slightly lower X^2 value for the 13:3 ratio. There was low heterogeneity among the four crosses indicating they could be from the same population. Possibly two loci

Table 13. Segregation for branching in F_2 populations.

Cross	Number of plants Observed (Expected) Branched	Unbranched	X^2 3:1	*P*
Manoa S[z] x 253229 B[y]	153 (149.3)	46 (49.8)	0.38	.50-.70
Manoa S x 281876 B	232 (210.0)	48 (70.0)	9.22	<.01**
Manoa S x 491092 B	149 (155.3)	58 (51.8)	1.01	.30-.50
Valma. S x 3777 B	78 (77.3)	25 (25.8)	0.03	.80-.90
273582 S x 281876 B	141 (127.5)	29 (42.5)	5.72	.01-.02*
236396 B x Manoa S	144 (141.8)	45 (47.3)	0.14	.70-.80
281876 B x 273579 S	117 (111.8)	32 (37.3)	0.99	.30-.50
281876 B x 491222 S	91 (77.3)	12 (25.8)	9.79	<.01**
491117 B x Valma. S	78 (77.3)	25 (25.8)	0.03	.80-.90
Pooled for all crosses	1183(1127.3)	320(375.8)	11.03	<.01**
Pooled for crosses without 281876	602 (600.8)	199(200.3)	0.01	.90-.95

Test for heterogeneity all crosses *P*

Total X^2	(9 df) = 27.34	
Pooled X^2	(1 df) = 11.03	
Heter. X^2	(8 df) = 16.31	.02-05*

Test for heterogeneity crosses without 281876

Total X^2	(5 df) = 1.59	
Pooled X^2	(1 df) = 0.01	
Heter. X^2	(4 df) = 1.58	.80-90

z Unbranched parent.
y Branched parent.

Table 14. 13:3 segregation for branching in F_2 populations.

Cross	Number of plants Observed (Expected) Branched	Unbranched	X^2 13:3	*P*
Manoa S[z] x 281876 B[y]	232 (227.5)	48 (52.5)	0.48	.30-.50
273582 S x 281876 B	141 (138.1)	29 (31.9)	0.33	.50-.70
281876 B x 273579 S	117 (121.1)	32 (27.9)	0.74	.30-.50
281876 B x 491222 S	91 (83.7)	12 (19.3)	3.40	.05-.10
Pooled	581 (571.2)	122(131.8)	0.90	.30-.50

Test for heterogeneity all crosses *P*

Total X^2 (4 df) = 4.95

Pooled X^2 (1 df) = 0.90

Heter. X^2 (3 df) = 4.05 .20-30

z Unbranched parent.

y Branched parent.

are interacting to cause the 13:3 ratio. The first locus has a dominant allele for branched (as found in the populations segregating 3:1) and is epistatic to a second locus with a dominant allele for unbranched. Thus 281876 is AAbb (branched), the other parents are aaBB (unbranched), the F_1 would be A-B- (branched), and the F_2 genotypes would be A-B- (branched), A-bb (branched), aaB- (unbranched), and aabb (branched). The crosses that gave a 3:1 ratio would be AABB (branched) x aaBB (unbranched). The proposed gene names and symbols are non-branching *b*-1 (replacing a in the above discussion), and branching *b*-2 (replacing b in the above discussion).

Bitterness

Segregating F_2 populations of two crosses of Manoa, a commercially grown lettuce in Hawaii, to the *L. serriola* accessions 190906 and 281876 were tested for bitterness by taste testing. Manoa was mild tasting with no bitter after-taste, while both *L. serriola* accessions were very acrid with a bitter after-taste. The F_2 was highly variable for this character ranging from the extremely bitter taste of the two *L. serriola* accessions to the mild, non-bitter taste of Manoa. Plants were only classified for the presence or absence of bitterness. They were classified non-bitter if they equaled Manoa in non-bitterness and were classified as bitter if they were more bitter than Manoa. Clearly, some bitter plants were more bitter than others, but it was not possible to evaluate degrees of bitterness.

Both populations seem to fit a bitter to non-bitter ratio of 15:1 (Table 15). Therefore, it appears at least two quantitative genes in the *L. serriola* lines cause bitterness.

Table 15. Bitterness segregation in F_2 populations.

Cross	Number of plants Observed (Expected) Bitter	Non-bitter	X^2 15:1	*P*
Manoa x 281876	136 (136.9)	10 (9.1)	0.09	.70-.80
Manoa x 190906	98 (101.3)	10 (6.8)	1.62	.20-.30

Abnormal leaf growth

The F_1's from all seven crosses involving 3006, which has extra lobes on both sides of the dorsal midrib where it branches into the first lobe, exhibited the extra lobes. This was most pronounced and occurred earliest on about the fourth or fifth leaf in the cross of the wide orbicular entire leaf Manoa x 3006, while in the cross of the narrow lanceolate entire leaf 273582 x 3006 and the reciprocal the trait was less noticeable and occurred later (about the seventh or eighth leaf). The other four crosses with three oblanceolate entire leaf plants (273574, 491222, and 253229) and one runcinate lobed leaf parent (281876) were all intermediate for extent and time of expression. Apparently leaf shape genes have a strong interaction with this character.

In the F_2 five of the seven populations gave a good fit to a ratio of three with the abnormal lobes to one without (Table 16). One F_2 population that did not fit a 3:1 ratio (Manoa x 3006) also had distorted ratios for other characters that were segregating (anthocyanin pigmentation and spines). The other population that did not fit a 3:1 ratio (253229 x 3006) had albino and chlorophyll deficient plants that died at the seedling stage, which may be the reason for the distorted ratio in this population.

Of the six parents that 3006 was crossed to, five had entire leaves and one (281876) had lobed leaves (3006 also had lobed leaves). In the F_2's of the crosses with entire leaved parents, only two types of plants were found, entire leaves without the extra lobe and lobed leaves with the extra lobe. In the cross with the lobed 281876 there

were also only two types, lobed leaves without the extra lobe and lobed leaves with the extra lobe. It seems the abnormal growth on the leaves is caused by an additional allele at the *U* locus tentatively named U^a. Three alleles are already known at this locus, *U* (lobed) and *u* (unlobed), and U^o (oakleaf) (Robinson et al., 1983). U^a is dominant to *U* as well as to *u*. It is not known whether U^a is dominant or recessive to U^o (which is dominant to *U*).

Table 16. Segregation for abnormal leaf lobes in F_2 populations.

Cross	Number of plants Observed (Expected) Abnormal	Normal	X^2 3:1	*P*
3006 x 273574	147 (152.3)	56 (50.8)	0.72	.30-.50
3006 x 273582	149 (148.5)	49 (49.5)	0.01	.90-.95
3006 x 281876[z]	69 (66.8)	20 (22.3)	0.30	.50-.70
491222 x 3006	99 (104.3)	40 (34.8)	1.06	.30-.50
273582 x 3006	118 (108.8)	27 (36.3)	3.15	.05-.10
Manoa x 3006	132 (116.3)	23 (38.8)	8.53	<.01**
253229 x 3006	182 (154.5)	24 (51.5)	19.58	<.01**

z 281876 is the only lobed leaf parent besides 3006.

Inheritance of previously reported characters

Anthocyanin pigmentation

Thirty-one F_2 populations had presence or absence of anthocyanin segregation recorded. There were three patterns of segregation all with presence of anthocyanin pigmentation dominant to no anthocyanin pigmentation. Twenty populations fit a 3:1, five populations fit a 9:7, and six populations fit a 54:10 ratio (Table 17). The 3:1 and 9:7 are normal segregation patterns (Robinson et al. 1983). However, the 54:10 is an unusual three gene ratio that has not been reported before. To verify the 54:10 ratio, F_3's were grown from twelve individual plants with anthocyanin from two of the populations. One F_3 (Manoa x 253339) had three families not segregate, five families segregated 3:1, and four families segregated 54:10. The other F_3 (Manoa x 3006) had six families not segregate and six other families segregate 3:1. There were no 15:1 or 9:7 ratios as would be expected in about half the families under the hypothesis of three genes. This suggests that the 54:10 ratio is the result of only one anthocyanin gene with 3:1 segregation, and that there is some predictable linkage between non-anthocyanic plants and reduced viability which can simulate a 54:10 ratio in certain segregating populations.

Table 17. Anthocyanin segregation in F_2 populations.

Crosses	Number of plants Observed (Expected) Anthocyanin	No	Ratio	X^2	P
3777 x 342517	93 (94.5)	33 (31.5)	3:1	0.24	.50-.70
491222 x 3006	113 (104.3)	26 (34.8)	3:1	0.40	.50-.70
273582 x 3006	106 (108.8)	39 (36.3)	3:1	0.28	.50-.70
274378 x 253229	116 (110.3)	31 (36.8)	3:1	1.20	.20-.30
253229 x 274378	119 (110.3)	28 (36.8)	3:1	2.78	.05-.10
Manoa x 281876	219 (219.8)	74 (73.3)	3:1	0.01	.90-.95
491117 x Valmaine	123 (124.5)	43 (41.5)	3:1	0.07	.70-.80
Manoa x 491092	151 (156.0)	57 (52.0)	3:1	0.64	.30-.50
253229 x 273579	131 (120.8)	30 (40.3)	3:1	3.48	.05-.10
491071 x 281876	60 (60.0)	20 (20.0)	3:1	0.00	>.99
236396 x Manoa	143 (141.8)	46 (47.3)	3:1	0.04	.80-.90
281876 x 273579	116 (113.3)	35 (37.8)	3:1	0.27	.50-.70
273582 x 281876	133 (127.5)	37 (42.5)	3:1	0.95	.30-.50
289015 x 491222	118 (122.3)	45 (40.8)	3:1	0.59	.30-.50
251798 x 274378	120 (130.5)	54 (43.5)	3:1	3.38	.05-.10
491071 x 236396	115 (123.0)	49 (41.0)	3:1	2.08	.10-.20
281876 x 274378	113 (107.3)	30 (35.8)	3:1	1.23	.20-.30
3006 x 273582	155 (150.8)	46 (50.3)	3:1	0.48	.30-.50
274378 x 3980	122 (129.0)	50 (43.0)	3:1	1.52	.20-.30
274378 x 271939	111 (102.3)	26 (34.3)	3:1	2.65	.10-.20
273574 x 190906	107 (115.3)	98 (89.7)	9:7	1.35	.20-.30
3006 x 273574	104 (114.2)	99 (88.8)	9:7	2.08	.10-.20
273574 x 289015	114 (111.9)	85 (87.1)	9:7	0.08	.70-.80
253229 x 183324	102 (90.6)	59 (70.4)	9:7	3.28	.05-.10
491092 x 183324	25 (28.7)	26 (22.3)	9:7	1.09	.20-.30
Manoa x 253229	167 (167.9)	32 (31.1)	54:10	0.03	.80-.90
Manoa x 190906	147 (151.0)	32 (28.0)	54:10	0.68	.30-.50
491222 x 3006	113 (117.3)	26 (21.7)	54:10	1.01	.30-.50
253229 x 3006	126 (124.9)	22 (23.1)	54:10	0.06	.80-.90
Manoa x 3006	132 (128.3)	20 (23.8)	54:10	0.70	.30-.50
281876 x 491222	97 (94.5)	15 (17.5)	54:10	0.42	.50-.70
Number of crosses					
20	2477 (2457)	799 (819)	3:1	0.65	.30-.50
5	452 (461)	367 (358)	9:7	0.40	.50-.70
6	788 (788)	146 (146)	54:10	0.00	>.99

Spines

Nineteen F_2 populations had segregation for presence or absence of spines recorded. Twelve populations segregated spined to non-spined 3:1 as expected (Robinson et al., 1983), while seven other populations did not fit a 3:1 ratio, all had a severe deficiency of non-spined plants (Table 18). One possible explanation for the deficiency of non-spined plants is some linkage between non-spined plants and a reduced viability similar to that of non-anthocyanin. Five of the seven crosses with non-spined deficiencies were with non-spined parents that had no normal 3:1 spine segregations, another cross (491222 x 3006) had the parent 491222 segregate for a 3:1 spine ratio in one cross (281876 x 491222) although there was a slight deficiency of non-spined plants. In these six crosses there could be another gene segregating for spines, although no gene ratio was found that could adequately explain the segregation. The seventh cross was Valmaine x 3777, Valmaine did have normal spine segregation in another cross (491117 x Valmaine). It is interesting that the other parent in this cross is the *L. aculeata* accession 3777, which has very dense spines, however, when 3777 was crossed to 342517 it gave a normal 3:1 spine ratio.

Table 18. Spine segregation in F_2 populations.

Crosses	Number of plants Observed (Expected) Spines	No	X^2 3:1	*P*
273574 x 190906	154(153.8)	51 (51.3)	0.001	.95-.99
3006 x 273574	83 (83.3)	28 (27.8)	0.15	.50-.70
190906 x 253229	143(150.0)	57 (50.0)	1.31	.20-.30
253229 x 3006	141(148.5)	57 (49.5)	1.52	.20-.30
3777 x 342517	94 (96.0)	34 (32.0)	0.17	.50-.70
253229 x 190906	100(109.5)	46 (36.5)	3.30	.05-.10
491117 x Valmaine	165(163.5)	53 (54.5)	0.06	.80-.90
491092 x 183324	162(161.3)	53 (53.8)	0.01	.90-.95
253229 x 273579	123(120.8)	38 (40.3)	0.17	.50-.70
281876 x 491222	89 (84.0)	23 (28.0)	1.19	.20-.30
183324 x 273579	119(115.5)	35 (38.5)	0.42	.50-.70
190906 x 3980	145(135.0)	35 (45.0)	2.96	.05-.10
Manoa x 190906	163(126.8)	18 (42.3)	>7.0	<.01**
491222 x 3006	123(104.3)	16 (34.8)	>7.0	<.01**
Manoa x 3006	138(116.3)	17 (38.8)	>7.0	<.01**
Manoa x 281876	120(102.0)	16 (34.0)	>7.0	<.01**
Valmaine x 3777	135(109.5)	21 (36.5)	>7.0	<.01**
Manoa x 491092	177(156.0)	31 (52.0)	>7.0	<.01**
491071 x 281876	71 (60.0)	9 (20.0)	>7.0	<.01**
Number of crosses				
12	1518 (1521)	510 (507)	0.01	.90-.95
7	1055 (909)	157 (303)	>7.0	<.01**

Leaf lobing

Seventeen F_2 populations had segregation for leaf lobing or non-lobing recorded. Twelve populations segregated 3:1 lobed to non-lobed as expected (Robinson et al., 1983), while two populations (190906 x 491092 and 491092 x 183324) had an excess of non-lobed plants and three populations (273582 x 281876, Manoa x 3006, and 253229 x 3006) had a deficiency of non-lobed plants (Table 19). Both populations with excess non-lobed plants had 491092 as a lobed parent. This accession is definitely lobed, but because of other leaf shape genes, it is not as pronounced as in other lobed parents. When 491092 was crossed with the wide leaf parent Manoa, the lobing was readily seen, however when it was crossed to the somewhat narrow leaf parents 190906 and 183324, it was more difficult to classify lobed plants. Therefore, the excess of non-lobed plants was probably caused by misclassification of genetically lobed plants. One of the severely deficient populations (253229 x 3006) had some albino and chlorophyll deficient plants, all of which died in the seedling stage. Another population deficient in non-lobed plants (Manoa x 3006) also had distorted segregation for anthocyanin and spines. This could indicate that linkage to a reduced viability gene may play some part in distorted ratios. The explanation why the third population (273582 x 281876) was deficient in non-lobed plants is unkown. It had normal segregation for anthocyanin and both parents segregated normally for lobing in other crosses.

Table 19. Leaf lobing segregation in F_2 populations.

Cross	Number of plants Observed (Expected) Lobed	Unlobed	X^2 3:1	*P*
3006 x 273574	147 (152.3)	56 (50.8)	0.72	.30-.50
3006 x 273582	149 (148.5)	49 (49.5)	0.01	.90-.95
3006 x 281876	69 (66.8)	20 (22.3)	0.30	.50-.70
491222 x 3006	99 (104.3)	40 (34.8)	1.06	.30-.50
273582 x 3006	118 (108.8)	27 (36.3)	3.15	.05-.10
Manoa x 281876	227 (221.3)	68 (73.8)	0.60	.30-.50
491117 x Valmaine	161 (164.3)	58 (54.8)	0.26	.50-.70
190906 x 491092	142 (156.0)	66 (52.0)	5.03	.02-.05*
491092 x 183324	133 (161.3)	82 (53.8)	19.80	<.01**
Manoa x 491092	154 (155.3)	53 (51.8)	0.04	.80-.90
491071 x 281876	57 (60.0)	23 (20.0)	0.60	.30-.50
281876 x 491222	86 (84.0)	26 (28.0)	0.19	.50-.70
281876 x 273579	119 (113.3)	32 (37.8)	1.17	.20-.30
273582 x 281876	147 (127.5)	23 (42.5)	11.93	<.01**
491117 x 3777	142 (150.0)	58 (50.0)	1.71	.10-.20
281876 x 274378	112 (107.3)	31 (35.8)	0.84	.30-.50
Manoa x 3006	132 (116.3)	23 (38.8)	8.53	<.01**
253229 x 3006	182 (154.5)	24 (51.5)	19.58	<.01**
Number of crosses				
12	1571 (1569)	521 (523)	0.01	.90-.95
2	175 (242)	148 (81)	>7.0	<.01**
3	461 (398)	70 (133)	>7.0	<.01**

Reflexed involucre

Eight F_2 populations had segregation for reflexed or non-reflexed involucres recorded (Table 20). All ten populations segregated 3:1 reflexed to non-reflexed as expected (Robinson et al., 1983).

Table 20. Involucre segregation in F_2 populations.

Cross	Number of plants Observed (Expected) Reflexed	No	X^2 3:1	*P*
3006 x 281876	70 (69.8)	23 (23.3)	0.003	.95-.90
Manoa x 281876	85 (86.3)	30 (28.8)	0.07	.70-.80
491117 x Valmaine	86 (82.5)	24 (27.5)	0.59	.30-.50
491092 x 183324	83 (84.0)	29 (28.0)	0.05	.80-.90
Manoa x 491092	47 (45.0)	13 (15.0)	0.36	.50-.70
491071 x 281876	51 (48.0)	13 (16.0)	0.75	.30-.50
281876 x 491222	62 (58.5)	16 (19.5)	0.84	.30-.50
Valmaine x 3777	79 (82.5)	31 (27.5)	0.59	.30-.50
All crosses	563 (556.5)	179(185.5)	0.30	.50-.70

Achene color

Six F_2 populations had segregation for dark and white achene color recorded (Table 21). All six segregated 3:1 dark to white achenes as expected (Robinson et al., 1983).

Table 21. Achene color segregation in F_2 populations.

Cross	Number of plants Observed (Expected) Dark	White	X^2 3:1	*P*
253229 x 491222	67 (66.8)	22 (22.3)	0.003	.95-.90
491117 x Valmaine	84 (81.8)	25 (27.3)	0.25	.50-.70
491222 x 3006	49 (49.5)	17 (16.5)	0.02	.80-.90
Manoa x 273574	68 (67.5)	22 (22.5)	0.02	.80-.90
273574 x 190906	56 (63.8)	29 (21.3)	3.77	.05-.10
3006 x 273574	136(127.5)	34 (42.5)	2.27	.10-.20
All crosses	460(456.8)	149(152.3)	0.09	.70-.80

Leaf tip shape

Three F_2 populations had segregation of pointed and round tipped leaves recorded (Table 22). All three segregated 3:1 pointed to round tip as expected (Robinson et al., 1983).

Table 22. Leaf tip shape segregation in F_2 populations.

Cross	Number of plants Observed (Expected) Pointed	Round	X^2 3:1	*P*
274378 x 3980	128 (129.0)	44 (43.0)	0.03	.95-.90
183324 x 273579	124 (115.5)	30 (38.5)	2.50	.10-.20
274378 x 271939	100 (102.8)	37 (34.3)	0.29	.50-.70
All crosses	352 (347.3)	111(115.8)	0.20	.50-.70

Linkage

The new characters reported in *L. sativa-L. serriola* group were tested for linkage to other segregating characters. No linkage was found between the genes for white pollen color and genes for anthocyanin, spines, branching, leaf lobing, or involucre type (Table 23). No linkage was found between the genes for branching and genes for anthocyanin, spines, or involucre type (Table 24). Linkage was found between branching and leaf lobing in four out of six crosses (Table 25). Linkage was not tested for bitterness because of its probable quanitative nature and few crosses. The new lobing allele was also not tested because it is not a new locus.

All four crosses that were significant for linkage between leaf lobing and branching had 281876 as one of the parents. In the discussion of branching, two genes were hypothesized as segregating in crosses between the branched 281876 parent and the unbranched parents. The accession 281876 was hypothesized to differ from other branched and unbranched parents used in this study by being homozygous recessive for a dominant non-branching locus. These results suggest that this is the locus that is linked to the leaf lobing locus. Crossover values ranged from .24-.36. However in the two largest populations the crossover values were .29 and .30 and the mean of all four crosses is .30.

Table 23. Tests for linkage between pollen color and other characters.

Loci compared[z] Crosses	Observed number each phenotype				Expected Ratio	Total	Linkage X^2	*P*
g; *wp*-1					9:3:3:1			
281876 x 273579	56	19	22	4		101	1.06	.30-.50
C; *wp*-1,*wp*-2					27:9:21:7			
Manoa x 281876	34	12	23	7		76	0.07	.70-.80
g; *wp*-1,*wp*-2					27:9:21:7			
281876 x 491222	36	6	24	4		64	0.03	.80-.90
273582 x 281876	40	12	25	6		83	0.08	.70-.90
sp; *wp*-1,*wp*-2					27:9:21:7			
Manoa x 281876	35	11	24	6		76	0.11	.70-.80
281876 x 491222	28	12	22	7		69	2.27	.10-.20
491071 x 281876	41	5	21	7		74	2.37	.10-.20
b-1,*b*-2; *wp*-1,*wp*-2					117:27:91:21			
281876 x 491222	32	8	24	5		69	0.08	.70-.80
273582 x 281876	46	6	29	2		83	0.08	.70-.80
u; *wp*-1,*wp*-2					27:9:21:7			
Manoa x 281876	34	12	25	5		76	0.75	.30-.50
273582 x 281876	39	13	23	8		83	0.01	.90-.95
281876 x 491222	30	10	22	7		69	0.01	.90-.95
er; *wp*-1					9:3:3:1			
3006 x 281876	54	20	22	8		104	0.00	>.99
er; *wp*-1,*wp*-2					27:9:21:7			
Manoa x 281876	39	11	35	9		94	0.05	.70-.80

z *g* = one of two complementary genes for anthocyanin; *wp*-1 = one of two complementary genes for yellow pollen; *C* = one of two complementary genes for anthocyanin; *wp*-2 = one of two complementary genes for yellow pollen; *sp* = spines; *b*-1 = one of two genes for branching; *b*-2 = one of two genes for branching; *u* = leaf lobing; *er* = erect involucre.

Table 24. Tests for linkage between branching and other characters.

Loci compared[z] Crosses	Observed number each phenotype				Expected Ratio	Total	Linkage X^2	*P*
sp; *b*-1					9:3:3:1			
491117 x Valmaine	39	16	15	5		75	0.12	.70-.80
er; *b*-1					9:3:3:1			
491117 x Valmaine	62	14	23	10		109	1.71	.10-.20
g; *b*-1					9:3:3:1			
236396 x Manoa	107	36	37	9		189	0.57	.30-.50
C; *b*-1					9:3:3:1			
491117 x Valmaine	87	36	24	19		166	2.92	.05-.10
C; *b*-1,*b*-2;					39:13:9:3			
281876 x 273579	87	27	30	5		149	1.80	.10-.20
273582 x 281876	108	32	25	3		168	1.51	.20-.30

z *g* = one of two complementary genes for anthocyanin; *C* = one of two complementary genes for anthocyanin; *sp* = spines; *b*-1 = one of two genes for branching; *b*-2 = one of two genes for branching; *er* = erect involucre.

Table 25. Linkage between leaf lobing and branching.

	Number observed (Expected)				39:13:9:3 Ratio	
	Branched		Unbranched		Linkage	
Crosses	Lobed	Unlobed	Lobed	Unlobed	X^2	*P*
Manoa x 281876	188(170.6)	44(56.9)	27(39.4)	21(13.1)	12.35	<.01**
	Crossover value = .30					
281876 x 491222	76 (62.8)	16(20.9)	4(14.5)	7 (4.8)	9.65	<.01**
	Crossover value = .24					
273582 x 281876	147(103.6)	14(34.5)	20(23.9)	9 (8.0)	6.02	.01-.02*
	Crossover value = .29					
281876 x 273579	97 (90.8)	20(30.3)	20(21.0)	12 (7.0)	5.84	.01-.02*
	Crossover value = .36					
				9:3:3:1 Ratio		
Manoa x 491092	108(116.4)	46(38.8)	41(38.8)	12(12.9)	1.09	.20-.30
491117 x Valmaine	99(101.3)	32(33.7)	32(33.7)	17(11.3)	2.22	.10-.20

Other characters observed to be segregating were also tested for linkage. No linkage was found between anthocyanin and leaf lobing, leaf lobing and spines, involucre type and spines, anthocyanin and involucre type, involucre type and leaf lobing, or anthocyanin and achene color (Table 26).

Table 26. Other character combinations tested for linkage.

Loci compared[z] Crosses	Observed number each phenotype				Expected Ratio	Total	Linkage X^2	*P*
C; *u*					9:3:3:1			
273582 x 281876	48	18	15	3		84	0.76	.30-.50
281876 x 273579	93	26	23	9		151	0.54	.30-.50
491117 x Valmaine	41	15	14	5		75	0.00	>.99
u; *sp*					9:3:3:1			
491222 x 3006	41	7	20	2		70	0.77	.30-.50
491117 x Valmaine	43	12	12	8		75	2.74	.05-.10
er; *sp*					9:3:3:1			
491117 x Valmaine	39	16	16	4		75	0.65	.30-.50
C; *er*					9:3:3:1			
491117 x Valmaine	66	14	19	10		109	3.31	.05-.10
er; *u*					9:3:3:1			
491117 x Valmaine	39	16	16	3		74	1.35	.20-.30
C; *w*					9:3:3:1			
491117 x Valmaine	42	14	15	4		75	0.12	.70-.80

z *C* = one of two complementary genes for anthocyanin; *sp* = spines; *b*-1 = one of two genes for branching; *b*-2 = one of two genes for branching; *er* = erect involucre; *u* = leaf lobing; *w* = dark achene color.

Eight out of 13 F_2 populations that segregated for anthocyanin and spines showed linkage (Table 27). There are two loci that control anthocyanin pigmentation (Robinson et al., 1983 and Table 17). Thus parents without anthocyanin can have either no genes for anthocyanin (and give 9:7 ratios) or one dominant locus (and give 3:1 ratios). Manoa, 342517, and Valmaine all gave 3:1 ratios and are thus dominant at one of the anthocyanin loci. However, since Manoa and 342517 both showed linkage with spines, and Valmaine did not, they must be homozygous at different loci. 491222, 273579, and 491071 also did not show linkage and thus should have the same anthocyanin gene as Valmaine. Two parents that gave 9:7 ratios (Table 17), 273574 and 183324, both also showed linkage as expected. Ryder (1983) concluded that one of the anthocyanin loci was linked to the spine locus based on only one F_2 population which had disturbed ratios for both anthocyanin and spines. These results with eight populations with linkage strongly confirm Ryder's conclusion. The crossover value was .36 for the only cross with undisturbed segregation for one anthocyanin locus and the spine locus. The three crosses segregating for both anthocyanin loci and the spine locus segregated normally for each trait, and had a crossover value of approximately .15. The lower crossover value but normal 9:7 anthocyanin to no anthocyanin ratio in the crosses segregating for both anthocyanin loci suggests that something has further suppressed crossovers between the linked anthocyanin locus and the spine locus. Perhaps other loci responsible for lower viability may also be linked, since many crosses segregating for spines and/or anthocyanin had disturbed segregations.

Table 27. Linkage between anthocyanin pigmentation and spines.

Crosses	Number observed (Expected) Anthocyanin Spines	Anthocyanin No spines	No anthocyanin Spines	No anthocyanin No spines	X^2	P
3777 x 342517	74 (70.9)	19(23.6)	19(23.6)	14 (7.9)	6.52	<.01**
	Crossover value = .36					
Manoa x 190906	90 (61.9)	1(20.6)	13(20.6)	6 (6.9)	24.51[z]	<.01**
491222 x 3006	99 (78.2)	14(26.1)	24(26.1)	2 (8.7)	0.46	.30-.50
Manoa x 3006	132 (88.3)	5(29.4)	8(29.4)	12 (9.8)	57.39[z]	<.01**
Manoa x 281876	199(164.2)	20(54.8)	46(54.8)	27(18.2)	53.14[z]	<.01**
253229 x 273579	82 (81.0)	32(27.0)	24(27.0)	6 (9.0)	0.79	.30-.50
281876 x 491222	79 (63.0)	18(21.0)	10(21.0)	5 (7.0)	1.74	.10-.20
Manoa x 491092	147(113.6)	2(37.9)	26(37.9)	27(12.6)	78.23[z]	<.01**
491117 x Valmaine	89 (93.4)	34(31.1)	34(31.1)	9(10.4)	0.77	.30-.50
491071 x 281876	53 (45.0)	7(15.0)	18(15.0)	2 (5.0)	0.04	.80-.90
				27:9:21:7 Ratio		
273574 x 190906	98 (86.5)	9(28.8)	56(67.3)	42(22.4)	32.78	<.01**
	Crossover value = .15					
3006 x 273574	96 (85.6)	8(28.5)	53(66.6)	46(22.2)	41.97	<.01**
	Crossover value = .13					
491092 x 183324	23 (21.5)	2 (7.2)	16(16.7)	10 (5.6)	6.23	.01-.02*
	Crossover value = .16					

z Crossover value could not be determined because of disturbed segregation ratio.

Inheritance of characters in crosses with L. saligna

There were two crosses between *L. saligna* and *L. sativa* (Table 6) and one cross between *L. saligna* and *L. aculeata* (Table 9) that produced viable F_1 achenes. The F_2 populations grown from these achenes had variable growth, ranging from large vigorous plants to small weak ones. More and more died as time passed, so the number of plants evaluated for different characters differs. Segregation was noted in these populations for the following characters: Lobed leaves, spines, anthocyanin, leaf tip shape, basal branching, pappus bristle cell width, anther sheath color, and achene beak to body ratio. The first four characters have been reported in *Lactuca* previously, although never in interspecific crosses with *L. saligna*, while the last four characters are new. Basal branching also segregated in the *L. sativa/ L. serriola* crosses (Table 13), but the last three characters are found only in *L. saligna*.

Previously reported characters

The characters leaf lobing, spines, anthocyanin pigmentation, and leaf tip shape have been reported on previously for *L. sativa-L.serriola* (Robinson et al., 1983). However, there are no reports on segregation for these characters in crosses with *L. saligna*.

The F_2 population from 11-1 x Manoa had 74 lobed and 15 unlobed plants, which fits a 3:1 ratio as found in crosses between *L. sativa* and *L. serriola*. The F_2 population of 491208 x 3777 had 11 lobed to 11 unlobed plants which did not fit a 3:1 ratio. However, six of the unlobed plants died before bolting and possibly could have been

genetically lobed plants that were misclassified. Leaf lobing was not recorded for the F_2 population of 491208 x Manoa.

Spine segregation in 491208 x Manoa was 39 with spines to 12 without, while 11-1 x Manoa segregated 70 with spines and 18 without. Both segregations fit a 3:1 ratio as found in crosses between *L. sativa* and *L. serriola*.

Anthocyanin pigmentation segregated 30 with and seven without in 491208 x Manoa, and 74 with and 22 without in 11-1 x Manoa (3777 has anthocyanin). Both segregations fit a 3:1 ratio as found in crosses between *L. sativa* and *L. serriola*.

Segregation for pointed and round leaf tips was only recorded in 11-1 x Manoa, where there were 59 pointed to 30 round which also fit a 3:1 ratio as found in crosses between *L. sativa* and *L. serriola*.

Thus, 491208 x Manoa had normal 3:1 segregations for spines and anthocyanin, and 11-1 x Manoa had the same for spines, anthocyanin, and leaf tip shape. Lobing, the only segregating character recorded in 491208 x 3777, did not give a normal segregation.

Branching

Two crosses between *L. saligna* and Manoa fit a 3:1 ratio for branched to unbranched, but had a slight deficiency of unbranched plants (Table 28). Because there was a deficiency in unbranched plants and because a 13:3 ratio was seen in crosses in the *L. sativa*-*L. serriola* group (Table 14), a 13:3 ratio was also tested. The two populations fit both ratios, but seemed to give a better fit to 13:3. It is interesting that 281876 which was originally received as *L.*

saligna, and has achenes similar in size and beak to body ratio as *L. saligna*, gave similar segregation ratios to *L. saligna* when crossed to non-branching plants. Perhaps there has been some introgression of genes into 281876 from *L. saligna*.

Table 28. Segregation for branching in F_2 populations with *L. saligna*.

Cross	Number of plants Observed (Expected) Branched	Unbranched	Ratio	X^2	*P*
491208 x Manoa	36 (33.0)	8 (11.0)	3:1	1.09	.20-.30
11-1 x Manoa	33 (30.0)	7 (10.0)	3:1	1.20	.20-.30
491208 x Manoa	36 (35.8)	8 (8.3)	13:3	0.01	.90-.95
11-1 x Manoa	33 (32.5)	7 (7.5)	13:3	0.04	.80-.90

Pappus bristles

Three crosses between *L. saligna* female parents and *L. sativa* and *L. aculeata* male parents were analyzed for pappus bristle width. This character can be used to distinguish *L. saligna* from all other species of subsection *Lactuca*. *Lactuca saligna* has bristles that are one cell in width, while all other species have bristles two cells in width. The F_1's all had pappus bristles which included two-cell width bristles indicating the *L. saligna* character is recessive. The F_2 in all three populations that produced achenes appeared to segregate 3:1 two-cell width bristles to primarily one-cell width bristles (Table 29).

Table 29. Segregation for pappus bristle cell width in F_2 populations with *L. saligna*.

One-cell parent	Two-cell parent	Number of plants Observed (Expected) Two-cell	One-cell	X^2 3:1	*P*
SAL 11-1	Manoa	28 (30.0)	12 (10.0)	0.53	.30-.50
SAL 491208	Manoa	27 (27.0)	9 (9.0)	0.00	>.99
SAL 491208	3777	11 (11.3)	4 (3.8)	0.02	.80-.90
Pooled		66 (68.3)	25 (22.8)	0.30	.50-.70

Test for heterogeneity *P*

Total X^2 (3 df) = 0.55

Pooled X^2 (1 df) = 0.30

Heter. X^2 (2 df) = 0.25 .80-.90

Anthocyanic anther sheaths

The crosses between *L. saligna* female parents and *L. sativa* and *L. aculeata* male parents were also analyzed for segregation of anthocyanic anther sheaths. The F_1's all had anthocyanic anther sheaths like the *L. saligna* parents indicating dominance for this trait. The F_2's in all three populations appeared to segregate three anthocyanic to one non-anthocyanic anther sheaths (Table 30). Two of these crosses also segregated 3:1 for anthocyanin (11-1 x Manoa 74-22; 491208 x Manoa 30-7). No plants were found that had anthocyanic anther sheaths but no anthocyanin in other plant parts. However, plants were found with non-anthocyanic anther sheaths that had anthocyanin in other plant parts in 491208 x 3777 (both anthocyanic parents) and in 11-1 x Manoa. Because of the low number of plants, no linkage analysis was done, but it appears that anthocyanin is only present in anther sheaths in plants that already have anthocyanin in other plant parts. Despite the low number of plants, the X^2 for heterogeneity was quite low indicating that all three segregating crosses could be from the same population.

Table 30. Segregation for anthocyanic anther sheaths in F_2 populations with *L. saligna*.

Anthocyanic anther sheath parent	Non-anthocyanic anther sheath parent	Number of plants Observed (Expected) Antho.	No antho.	X^2 3:1	*P*
SAL 11-1	Manoa	28 (28.5)	10 (9.5)	0.03	.80-.90
SAL 491208	Manoa	30 (27.8)	7 (9.3)	0.73	.30-.50
SAL 491208	3777	11 (11.3)	4 (3.8)	0.02	.80-.90
Pooled		69 (67.5)	21 (22.5)	0.13	.70-.80

Test for heterogeneity *P*
Total X^2 (3 df) = 0.82
Pooled X^2 (1 df) = 0.13
Heter. X^2 (2 df) = 0.69 .70-.80

Achene beak length to body length ratio

The three crosses between *L. saligna* and *L. aculeata* and *L. sativa* were also analyzed for achene beak to body length ratios. *Lactuca saligna* has a noticeably longer beak in relation to its body than do *L. sativa* or *L. aculeata*. Since the bodies are approximately the same length, *L. saligna* has a higher ratio of the two measurements (Table 31). In the F_1's the beak lengths were about the same or shorter than the short parent, while the body lengths were all longer than either parent (possibly showing heterosis). Thus the ratios in the F_1's were all smaller than the small parent.

The F_2 plants were classified as low if their ratio was equal to or less than the low ratio parent, and high if their ratio exceeded the low ratio parent (Table 12). Only one population (11-1 x Manoa) fits a ratio segregation pattern of 3 low to one high quite well. The cross of 491208 x Manoa did not fit a 3:1 ratio. This was probably

caused by one or more of the following reasons: the low number of plants, the reduced fertility of the interspecific cross, environmental influences, or that this character may not be controlled by one major gene, but quantitatively by several loci. The cross of 491208 x 3777 did not have any high ratio plants, perhaps for the same reasons. Therefore, inheritance of this character can not be fully explained at this time.

Table 31. Average achene beak length, achene body length, and ratio between beak and body for parents and F_1's.

Parents and F_1	Beak length	Body length	Ratio
SAL 11-1	5.4	3.1	1.7
Manoa	4.4	3.3	1.3
F_1	4.5	4.0	1.1
SAL 491208	5.8	3.0	1.9
Manoa	4.4	3.3	1.3
F_1	3.9	3.7	1.1
SAL 491208	5.8	3.0	1.9
3777	3.5	3.0	1.2
F_1	3.0	3.2	0.9
SAL 491208	5.8	3.0	1.9
236396	4.6	3.9	1.2
F_1[z]	4.0	4.3	0.9

z F_1 consisted of only 3 achenes.

Table 32. Segregation for achene beak length to body length ratio in F_2 populations with *L. saligna*.

High beak ratio parent	Low beak ratio parent	Number of plants Observed (Expected) Low	High	X^2 3:1	*P*
SAL #11-1	Manoa	22 (21.0)	6 (7.0)	0.19	.50-.70
SAL 491208	Manoa	28 (22.5)	2 (7.5)	5.38	.02-.05*
SAL 491208	3777	6 (4.5)	0 (1.5)	1.88	.10-.20
Pooled		56 (48.0)	8 (16.0)	4.33	.02-.05

Test for heterogeneity — *P*

Total X^2 (3 df) = 7.45

Pooled X^2 (1 df) = 4.33

Heter. X^2 (2 df) = 3.12 .20-.30

SUMMARY AND CONCLUSIONS OF INHERITANCE STUDY

The previously unreported characters white pollen color, basal branching, extra leaf lobe growth, and bitterness were seen to segregate in F_2 populations within the *L. sativa-L. serriola* complex.

One accession (PI 281876) was observed to have white pollen instead of the normal yellow. Pollen color segregated yellow to white in 9:7 and 3:1 ratios indicating that two complementary loci control this trait. The proposed name and gene symbols for these loci are white pollen-1 *wp*-1, and white pollen-2 *wp*-2.

The inheritance of basal branching has not been previously reported. Basal branching segregated branched to unbranched in 3:1 and 13:3 ratios. The evidence suggests one locus with a dominant allele for branching epistatic to a second locus with a dominant allele for non-branching. The second locus appeared to be linked to the leaf lobing locus with an approximate crossover value of .30. The proposed name for these loci are non-branching *b*-1, and branching *b*-2.

An extra leaf lobe growth on the dorsal side of leaves in Ac 3006 segregated three with this trait to one without. This character seems to be caused by a new dominant allele (U^a) at the previously reported leaf lobing locus (*u*).

The acrid, bitter taste found in wild lettuce accessions segregated bitter to non-bitter in a ratio approximating 15:1 suggesting at least two loci control this probably quantitative trait.

Linkage was observed between one branching locus and the leaf lobing locus. No linkage was found between pollen color or branching

and previously reported loci for anthocyanin pigmentation, spines, achene color, leaf tip shape, and involucre position. There was no additional linkage found among the previously reported characters, except between spines and anthocyanin with a crossover value of approximately .15 in crosses segregating for both anthocyanin loci, and a crossover value of .36 in one cross segregating for a single anthocyanin loci. This linkage confirms the suspicion of Ryder (1983).

F_2 populations of crosses between *L. saligna* and the *L. sativa-L. serriola* complex segregated for the previously unreported characters basal branching, pappus bristle width, anthocyanic anther sheaths, and achene beak length to body length ratio.

Branching seemed to segregate 13:3 as was reported above in crosses within the *L. sativa-L. serriola* complex. Pappus bristle width segregated 3:1 two-cell width to one cell width, which indicated one major locus controls this trait. Anthocyanic anther sheaths segregated three with anthocyanin to one without, which suggests one locus controls this trait. Achene beak to body ratio appeared in one cross to segregate three high to one low, but this appears to be a quantitative trait caused by several interacting loci. Anthocyanin pigmentation, spines, leaf lobing, and leaf tip shape all appeared to segregate in normal 3:1 ratios.

All members of the *L. sativa-L. serriola* complex gave mostly normal segregation in F_2 interspecific populations. This is more evidence for the assertion that they may in fact be one species.

Thus, desirable genes from any of the members could easily be incorporated into the commercially important *L. sativa*.

Crosses between *L. saligna* and members of the *L. sativa-L. serriola* complex also gave mostly normal segregation in F_2 interspecific populations, so despite the lower fertility of these crosses, these results suggest that other potentially important genes for such traits as disease resistance and stress tolerance could also be transferred from *L. saligna* to the commercially important *L. sativa*.

LITERATURE CITED

Abrams, L. and R.S. Ferris. 1940. *Lactuca*. Ill. Flora of the Pacific States. 4:590-594.

Babcock, E.B., G.L. Stebbins, and J. A. Jenkins. 1937. Chromosomes and phylogeny in in some genera of the *Crepidinae*. Cytologia, Fujii jub. 188-210.

Bailey, L.H. 1976. Hortus Third. Macmillan Publishing Company. 631.

Britton, N.L. 1913. Chicory family. Ill. Flora of the N. U.S., Can., and the Brittish Possesions. 3:317-321.

Brown, P.R., and R.W. Michelmore. 1988. The genetics of corky root resistance in lettuce. Phytopathology. 78:1145-1150.

Carr, G.D. 1975. Chromosome evolution and aneuploid reduction in *Calycadenia pauciflora*. Evolution. 29:681-699.

Cohen, O. and A. Liston. 1986. *Lactuca aculeata*. Rotem. 21:72-78,93-94.

Cronqvist, A. 1955a. Phylogeny and Taxonomy of the *Compositae*. American Midland Naturalist. 53:478-512.

Durst, C.E. 1930. Inheritance in lettuce. Illinois Agr. Exp. Stat. Bull. 356:239-341.

Eenink, A.H., R. Groenwold, and F.L. Dieleman. 1982. Resistance of lettuce (*Lactuca*) to the leaf aphid *Nansonovia ribis nigri*. 1. Transfer of resistance from *L. virosa* to *L. sativa* by interspecific crosses and selection of resistant breeding lines. Euphytica 31:291-300.

Einset, J. 1944. Cytological basis for sterility in induced autotetraploid lettuce. Amer. Jour. Bot. 31:336-342.

Ernst-Schwarzenbach, M. 1936. Fertilitat, photoperiodismus und genetik von *Lactuca sativa* L. Der Zuchter. 8:11-21.

Ferakova, V. 1976. CLXIX. *Compositae*. In Flora Europaea. T.G. Tutin, V.H. Heywood, N.A. Burges, D.M. Moore, D.H. Valentine, S.M. Walters, and D.A. Webb (eds.). 4:103,328-331.

Ferakova, V. 1977. The genus *Lactuca* L. in Europe. Univerzita Komenskeho, Czechoslovakia.

LITERATURE CITED (Continued)

Gleason, H.A. (ed.) 1952. Composite family, *Lactuca* L. lettuce. New Britton and Brown Illustrated Flora. 3:535-538.

Gleason, H.A. and A. Cronqvist. 1955. Vascular plants of the Pacific Northwest. Part 5. *Compositae*. Seattle.

Globerson, D., D. Netzer, and J. Sacks. 1980. Wild lettuce as a source for improving cultivated lettuce. In Eucarpia Meeting on Leafy Vegetables. Littlehampton, U.K. 86-97.

Guedes, A.C. and D.J. Cantliffe. 1980. Germination of lettuce seeds at high temperature after seed priming. Jour. Amer. Soc. Hort. Sci. 105(6):777-781.

Hegi, G. 1928-1929. Illustrierte Flora Von Mittel-Euopa. Munchen. 1113-1134.

Immer, F.R. 1930. Formulae and tables for calculating linkage intensities. Genetics. 15:81-98.

Jeffrey, C. 1966. Notes on *Compositae* I. The *Cichorieae* in east tropical Africa. Kew Bull. 18(3):427-486.

Jeffrey, C. 1975. *Lactuca* L. Flora of Turkey and the East Aegean Islands. 5:776-782.

Johnson, A.G., S.A. Laxton, I.R. Crute, P.L. Gordon, and J.M. Norwood. 1978. Further work on the genetics of race specific resistance in lettuce to downy mildew (*Bremia lactucae*). Ann. App. Biol. 89:257-264.

Kesseli, R.V. and R.W. Michelmore. 1986. Genetic variation and phylogenies detected from isozyme markers in species of *Lactuca*. Jour. of Heredity. 77:324-331.

Kesseli, R.V., O. Ochoa, and R.W. Michelmore. 1991. Variation in RFLP loci in *Lactuca* spp. and origin of cultivated lettuce. Genome. 34:430-433.

Koster, J.T. 1976. The *Compositae* of New Guinea V. Blumea 23:163-175.

Lindkvist, K. 1960a. Cytogenetic studies in the *serriola* group of *Lactuca*. Hereditas. 46:75-151.

Lindkvist, K. 1960b. Inheritance studies in lettuce. Hereditas. 46:387-470.

LITERATURE CITED (Continued)

Lindkvist, K. 1960c. On the origin of cultivated lettuce. Hereditas. 46:319-349.

Linnaeus, C. 1753. Species Plantarum. 2:795.

Mather, K. 1951. The Measurement of Linkage in Heredity. Methuen & Co. Ltd., London, U.K.

Matsumoto, E. 1991. Interspecific somatic hybridization between lettuce (*Lactuca sativa*) and wild species *L. virosa*. Plant Cell Reports. 9:531-534.

Moore, R.J. (ed.). 1965-1985. Index to plant chromosome numbers. Oosthoek Uitgeversmaatschappij, Utrecht, Netherlands.

Oliver, G.W. 1910. New methods of plant breeding. U.S. Bur. Plant Ind. Bull. 167.

Radloff, H.W. 1961. *Lactuca canadensis, L. graminifolia L. hirsuta, L. ludoviciana* - four species or one? Amer. Jour. Bot. 48:549.

Reinink, K., I. Vries, and R. Groenwold. 1988. Interspecific crosses in lettuce within section *Lactuca*. Prophyta. 42(6):156.

Robinson, R.W., J.D. McCreight, and E.J. Ryder. 1983. In Plant Breeding Reviews. J. Janick (ed.). A.V.I. Publishing Company, Westport, Ct. 1:267-293.

Ross-Craig, S. 1963. Drawings of British Plants. Pl. 31-34. London:G. Bell and Sons Ltd.

Ryder, E.J. 1963a. A gene for depth of corolla cleft in the lettuce flower. Vegetable Improvement Newsletter. 5:5-6.

Ryder, E.J. 1963b. An epistatically controlled pollen sterile in lettuce. Proc. Amer. Soc. Hort. Sci. 83:585-589.

Ryder, E.J. 1965. The inheritance of five leaf characters in lettuce. Proc. Amer. Soc. Hort. Sci. 86:457-461.

Ryder, E.J. 1970. Inheritance of resistance to common lettuce mosaic. Jour. Amer. Soc. Hort. Sci. 95:378-379.

Ryder, E.J. 1971. Genetic studies in lettuce. Jour. Amer. Soc. Hort. Sci. 96:826-828.

LITERATURE CITED (Continued)

Ryder, E.J. 1975. Linkage and inheritance in lettuce. Jour. Amer. Soc. Hort. Sci. 100:346-349.

Ryder, E.J. 1979. Lettuce. Leafy Salad Vegetables. A.V.I. Publishing Company Incorporated. 13-94.

Ryder, E.J. 1983. Inheritance, linkage, and gene interaction studies in lettuce. Jour. Amer. Soc. Hort. Sci. 108(6):985-991.

Ryder, E.J. 1986. Lettuce breeding. In Bassett, M.J. (ed). Breeding Vegetable Crops. A.V.I. Publishing Company Incorporated. 436-476.

Ryder, E.J. 1988. Early flowering in lettuce as influenced by a second flowering time gene and seasonal variation. Jour. Amer. Soc. Hort. Sci. 113(3)456-460.

Ryder, E.J. 1989. Studies of three new genes, linkage, and epistasis in lettuce. Jour. Amer. Soc. Hort. Sci. 114(1):129-133.

Ryder, E.J. and A.S. Johnson. 1974b. Mist depollination of lettuce flowers. HortScience. 9:584.

Shih, C. 1988. Revision of *Lactuca* L. and two new genera of tribe *Lactuceae* (*Compositae*) on the mainland of Asia. Acta Phytotaxonomica Sinica. 26(6):418-428.

Statistics of Hawaiian Agriculture. 1990.

Stebbins, G.L. 1936. Two new species of *Lactuca* from tropical Africa. Bulletin Jardin Botanique National Belgique. 14:223-226.

Stebbins, G.L. 1937. The scandent species of *Prenanthes* and *Lactuca* in Africa. Bull. Jard. Bruxelles. 14:333-352.

Stebbins, G.L. 1953. A new classification of the tribe *Cichorieae*, family *Compositae*. Madrono. 12:65-81.

Stebbins, G.L., J.A. Jenkins, and M.S. Walters 1953. Chromosomes and phylogeny in the *Compositae*, tribe *Cichorieae*. Univ. Calif. Publ. Bot. 26:401-430.

Tackholm, V. 1956. Students' Flora of Egypt. Cairo.

LITERATURE CITED (Continued)

Thompson, R.C. 1943. Further studies on interspecific genetic relationships in *Lactuca*. Jour. Agric. Res. 66(1):41-48.

Thompson, R.C., and E.J. Ryder 1961. Description and pedigrees of nine varieties of lettuce. U.S.D.A. Tech. Bull. 1244:1-19.

Thompson, R.C., T.W. Whitaker, W.F. Kosar 1941. Interspecific genetic relationships in *Lactuca*. Jour. Agric. Res. 63(2):91-107.

Tuisl, G. 1977. *Lactuca*. In Flora Iranica. K.H. Rechinger (ed.) no. 122:185-196, tab. 124-133,201.

Whitaker, T.W. 1944. The inheritance of chlorophyll deficiencies in cultivated lettuce. Jour. of Heredity. 35:317-320.

Whitaker, T.W. 1950. The genetics of leaf form in cultivated lettuce. I. The inheritance of lobing. Proc. Amer. Soc. Hort. Sci. 56:389-394.

Whitaker, T.W. 1968. A chlorophyll-deficient mutant in lettuce. Vegetable Improvement Newsletter. 10:5.

Whitaker, T.W. and I.C. Jagger 1939. Cytogenetic observations in *Lactuca*. Jour. Agric. Res. 58(4):297-306.

Whitaker, T.W. and D.E. Pryor. 1941. The inheritance of resistance to powdery mildew (*Erysiphe cichoracearum*) in lettuce. Phytopath. 31:534-540.

Whitaker, T.W. and R.C. Thompson 1941. Cytological studies in *Lactuca*. Bull. Torrey Bot. Club 68:388-394.

Vries, I.M. De 1990. Crossing experiments of lettuce cultivars and species (*Lactuca* sect. *Lactuca, Compositae*). Pl. Syst. Evol. 171:233-248.

Vuillemier, B.S. 1973. The genera of *Lactuceae* (*Compositae*) in the southeastern United States. Jour. Arnold Arbor. 54, 1:42-93.

Zohary, D. 1991. The wild genetic resources of cultivated lettuce (*Lactuca sativa* L.). Euphytica 53:31-35.

Zohary, D. and M. Hopf. 1988. Domestication of plants in the old world. Oxford University Press, New York, N.Y. 170.

LITERATURE CITED (Continued)

Zoku, A. 1965. *Lactuca*. In Flora of Japan. J. Ohwi (ed.) English version F.G. Meyer and E.H. Walker (eds.) Smithsonian Inst. Washington D.C. 928-929.

Zink, F.W. and J.E. Duffus. 1970. Linkage of turnip mosaic virus susceptibility and downey mildew, *Bremia lactucae*, resistance in lettuce. Jour. Amer. Soc. Hort. Sci. 95:420-422.

Zink, F.W. and J.E. Duffus. 1973. Inheritance and linkage of turnip mosaic virus and downey mildew (*Bremia lactucae*) reaction in *Lactuca serriola*. Jour. Amer. Soc. Hort. Sci. 98:49-41.

Zitter, T.A. and V.L. Guzman. Evaluation of cos lettuce crosses, endive cultivars, and *Cichorium* introductions for resistance to bidens mottle virus. Plant Dis. Reporter. 61:767-770.